PROCESSED COLLAGEN GRAFT FOR THE TREATMENT OF CORNEAL LESIONS IN DOGS

T. A. RAJI

Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2006

Department of Veterinary Surgery and Radiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

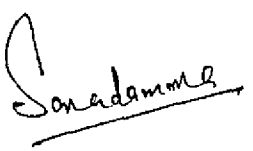
I, hereby declare that this thesis entitled **"PROCESSED COLLAGEN GRAFT FOR THE TREATMENT OF CORNEAL LESIONS IN DOGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Mannuthy
30-9-2006

T. A. Raji

CERTIFICATE

Certified that this thesis entitled " **PROCESSED COLLAGEN GRAFT FOR THE TREATMENT OF CORNEAL LESIONS IN DOGS**" is a record of research work done independently by **T. A. Raji** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



Dr. T. Sarada Amma
(Chairperson, Advisory Committee)
Associate Professor and Head
Department of Veterinary Surgery and Radiology
College of Veterinary and Animal Sciences
Mannuthy

Mannuthy
30 -9 -2006

CERTIFICATE

We, the undersigned members of the Advisory Committee of **T. A. Raji**, a candidate for the degree of Master of Veterinary Science in Veterinary Surgery, agree that the thesis entitled **"PROCESSED COLLAGEN GRAFT FOR THE TREATMENT OF CORNEAL LESIONS IN DOGS"** may be submitted by **T. A. Raji** in partial fulfillment of the requirement for the degree.

30/9/2006

Dr. T. Sarada Amma
(Chairperson, Advisory Committee)
Associate Professor and Head
Department of Veterinary Surgery and Radiology
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur.

30/09/06

Dr. K. Rajankutty
Associate Professor
Department of Veterinary Surgery
and Radiology
(Member)

30/9/06

Dr. Syam. K. Venugopal
Assistant Professor (Sr. scale)
Department of Veterinary
Surgery and Radiology
(Member)

30/09/06

Dr. G. Krishnan Nair
Associate Professor and Head
Department of Veterinary
Microbiology
(Member)

14/11/06

External Examiner

Dr. C. RAMANI, Ph.D.,
Associate Professor,
Dept. of Surgery & Radiology
Madras Veterinary College,
Chennai-7

ACKNOWLEDGEMENTS

With immense pleasure, I depict my sincere and heartfelt gratitude to the Chairperson of my advisory committee, Dr.T. Sarada Amma, Associate Professor and Head, Department of Veterinary Surgery and Radiology, for the meticulous guidance, keen interest and supervision, affectionate encouragement and guidance, persuasion, critical comments and endless help offered to me from the initiation of my work to successful completion of the manuscript.

I am very much grateful, to Dr. K. Rajankutty, Associate Professor, Department of Veterinary Surgery and Radiology, for his timely advice, suggestions and support, for his incessant help rendered during various stages of my work till the very end, which has been of great help at the time of difficulties.

There are no words to pay my respect and deep sense of gratitude to Dr.Syam. K. Venugopal, Assistant Professor (Sr.scale), Department of Veterinary Surgery and Radiology, for sparing his time to employ his technical skill throughout the work, expert advice and suggestions that led me to this topic, constructive criticism and ardent encouragement, whenever I needed.

I am much obliged to Dr. G. Krishnan Nair, Associate Professor, Department of Veterinary Microbiology, for his valuable suggestions, and wholehearted approach throughout the study and moreover for the cooperation rendered for microbiological investigation.

I am extremely grateful to the help and guidance provided by the Dr. P. K. Sehgal, Deputy Director & Head and Dr. Hadassah Victor, Senior Research Fellow, Bio-Products Lab, Central Leather Research Institute, Adyar along with providing the required material for the research work.

I deem it my privilege to express my gratitude to Dr. C.B. Devanand, Dr. K. D. John Martin and Dr. M.K. Narayanan, Assistant Professors, Department of Veterinary

Surgery and Radiology, for their whole-hearted cooperation, kindness and timely help and guidance during the course of my study.

I express my extreme sense of gratitude to the Dean, College of Veterinary and Animal Sciences, Mannuthy, for providing me the necessary facilities for carrying out this work.

I cherish the generous help, moral support and constant encouragement rendered by my colleagues Dr. B. Venkateswaralu, Dr. M. Ranjith Mohan and Dr. Soumya Ramankutty. The uniqueness of their support helped me so immensely all throughout the study, that I cannot express my words of gratitude.

The mental support and sustained encouragement rendered by Dr. Laiju M. Philip, Dr. B. Julie, Dr. Philip Varghese, Dr. K. M. Dileep Kumar and Dr. Reji Varghese is gratefully acknowledged. I am immensely grateful to Dr. Tessy Mathew and Dr. N. S. Jineshkumar for the selfless help and friendly support offered by them whenever I needed it most.

I am much obliged to my friends Dr. Sambhunath Chaudhary, Dr. R. Rajagopal, Dr. G. Sunil, Dr. M. G. Jibi, Dr. Ambili, and Dr. A. R. Ranjini for offering assistance and spending their time which ensured the successful completion of this study.

With immense gratitude and fondness, I remember my friends, Dr. K. B. Sumena, , Dr. A. Manjusha, Dr. Raseena Karim, Dr. Preethymol Joseph, Dr. P. K. Deepa, Dr. K.Jaibi, Dr. Ranimol Antony Dr. Lekha Chako, Dr. V. R. Bini,, Dr. C. Ananthalekshmy, Dr. T. X. Seena, Dr. K.. R. Mejo, Dr. Kavitha Rajagopal, , Dr. K. Jasmine Rani, Dr. Biya Ann Leon for their warm friendship, affectionate encouragement and timely help and suggestions that helped me to carry out this work to fruitfulness.

I express my sincere gratitude to the students of 2000 batch and 2001 batch, for their help and co-operation extended during my research work.

I am thankful to Mrs. K. Indira Devi,, Radiographer, Department of Veterinary Surgery and Radiology for the personal interest and assistance offered during the period of study.

I express my sincere gratitude to Smt. A. Mary, Smt. P. S. Beena, Smt. K. Lathika and Smt. T. V. Rajani for the timely assistance for the conduct of the work.

I am also grateful to all the teaching and non-teaching staffs of the Veterinary Hospitals at Mannuthy and Kokkalai for their sincere help and co-operation during the entire work.

I wish to place on record a note of thanks to Kerala Agricultural University for granting me the KAU Junior fellowship.

I am deeply indebted to my most beloved parents and brother, for their love, prayers, constant support and encouragement without which I would not have been able to complete this work successfully.

Above all, from the depth of my heart, I offer salutations to 'The God Almighty' who had always been a helping hand, walking by my side, leading me through most difficult situations up to this successful ending.

T. A. Raji

CONTENTS

LIST OF TABLES

LIST OF PLATES

Introduction

1. INTRODUCTION

Corneal affections are the major causes of visual handicap in both human beings and animals. Common corneal lesions include ulceration, perforation, staphyloma and different degrees of opacification and pigmentation. Traditionally, the treatment for such conditions in dogs includes topical medications, scarification or suturing along with routine medicinal application. Surgical methods are seldom used. Whatever may be the method of treatment adopted, it requires a long time to achieve clarity of the cornea.

The process of healing of cornea is entirely different from that of other tissues and it is partly based on the thickness to which the defect is involved and the severity of the condition. Clinical use of porcine small intestinal submucosa (Lewin, 1999) and equine amniotic membrane (Barros *et al.*, 2005) for the repair of full thickness defects of cornea in dogs had been reported.

Recently, to promote the corneal tissue healing use of collagen shield has been recommended in human beings (Willoughby *et al.*, 2002). A few experimental evaluations have been reported in cat (Shaker *et al.*, 1989) and rabbit (Robin *et al.*, 1990).

The importance of this study lies in the fact that it reduces the time of healing of corneal wound (Willoughby *et al.*, 2002) and is feasible even at the field level with minimum facilities. The added advantage of the technique is avoidance of frequent medication during the postoperative period in intraocular surgeries as it acts as a sustained drug delivery system. The collagen diskettes for ophthalmic use, is being produced in the Central Leather Research Institute (CLRI) and its clinical use is in trial.

Hence the study is undertaken with an objective of evaluating the efficacy of collagen diskette as an onlay graft for the treatment of various corneal lesions in dogs.

Review of Literature

2. REVIEW OF LITERATURE

2.1. ANATOMY OF THE CANINE EYE AND CORNEA

Krawitz (1963) described that the incomplete orbit may be a predisposing factor for proptosis or prolapse of the globe and the occurrence of retro-orbital abscesses in dogs and reported a higher incidence of ocular problems in short-headed dogs. It was also stated that cornea is the only tissue whose design and architecture makes it transparent and that its entire function depends on transparency which is related to hydration and increased hydration leads to scattering of light resulting in opacity.

Shivley and Epling (1970) conducted an electron microscopic study of the canine cornea and reported that corneal epithelium was a continuation of the conjunctival epithelium. This study revealed the continuity of conjunctival substantia propria into the cornea as the cephalic obliquely oriented layer of collagen bundles and that the deep stroma, descemet's membrane and the endothelium were of mesodermal in origin. The dual origin of the corneal layers was pointed out, that could account for some manifestations of corneal disease.

Samuelson (1991) reported that the lack of blood vessels, non-keratinized surface epithelium maintained by a preocular moisture film, lack of pigmentation and size and organization of collagen fibrils were responsible for the transparency of the cornea.

Whitley (1991) explained that most of the corneal stroma was composed of collagen arranged in lamellar pattern. Keratocytes and fibroblasts were seen within and in between them. The special arrangement of the collagen fibrils permit 99% of the light entering the cornea to pass without

scatter and he stated that any rearrangement of the precise collagen organization will result in opacity of the cornea.

Wilcock (1993) described the factors contributing to corneal clarity *viz.,* regular, non-keratinized and non-pigmented surface epithelium, an avascular, cell poor stroma composed of collagen fibrils in lamellar pattern and a high degree of stromal dehydration, effected by the endothelium.

Transprency of the cornea was explained to be brought about by non-keratinized epithelium, precise organization of small diameter collagen fibrils, endothelial pump function and the absence of blood vessels and pigment (Grevan, 2000).

2. 2. PHYSIOLOGY OF THE CANINE CORNEA

Krawitz (1963) described tears as an important source of nutrition for the cornea and a reduced supply of the tears or corneal film will bring about a pathologic change. It was opined that, an increase in the number of leucocytes between the basal cells during inflammation, accounts for the corneal haze occurring in keratitis. Continued good health of the cornea was considered to be depended upon its ability to maintain a dynamic equilibrium with its environment and any change in the adnexal structures, tear or aqueous humor, which cause unfavourable environmental conditions, which may affect the cornea.

According to Gum (1991), the energy requirement of the cornea in the form of ATP was furnished mainly by the aqueous humor and was mainly utilized to maintain the state of dehydration. Under stressful conditions, glycogen storage in the epithelium acts as the energy source and therefore if these stores become depleted, normal healing of the epithelium and cellular locomotion over the surface will be inhibited.

2.3. BREED PREDISPOSITION

Bedford (1982) observed increased occurrence of complete prolapse of the globe in brachycephalic breeds of dogs even with negligible amount of force, which may be complicated by corneal desiccation and exposure keratitis.

Startup (1984) observed that the brachycephalic breeds, particularly those with large corneal areas, and especially Pekingese and Pug, were prone to corneal injury and subsequent infection. It was opined that, certain strains within these breeds were even more susceptible due to inherited corneal insufficiency and a lack of protective eye consciousness (poorly developed corneal reflex and a lack of corneal sensitivity), which may predispose these breeds to the occurrence of neurotrophic ulcers. It was described that damage or deficiencies of the ophthalmic branch of trigeminal nerve affect the sensitivity of the cornea and result in epithelial degeneration. Lack of sensation, oedema and epithelial erosion leads to ulceration. It was stated that in some of the brachycephalic breeds, there is the possibility of nerve deficiencies, particularly affecting the central corneal areas.

From the studies conducted by Whitley *et al.* (1995) it was clear that certain breeds were more prone to develop some form of eye disorders. But in some of them an inheritance pattern has not been proved. He reported that various types of corneal disorders were common in Belgian Sheepdog, Chihuahua, Chow chow, Cocker Spaniel (American), Collies, Dachshund, Dalmatian, English Setter, English Springer Spaniel, Fox Terrier, German Shepherd Dog, Golden Retriever, Hounds, King Charles Spaniel, Labrador Retriever, Lhasa Apso, Mastiff, Pekingese, Pointer, Poodle and Pug. Among them a majority of disorders like, corneal dystrophy, exposure keratopathy, lagophthalmos, exophthalmos, euryblepharon, nasal fold trichiasis and

associated corneal problems, pigmentary keratitis, traumatic proptosis, trichiasis and ulcerative keratitis were found to be affecting pug.

2.4. OPHTHALMIC EXAMINATION

2.4.1. Gross Examination

Felchle and Urbanz (2001) described the various techniques of examination of eye in animals which should start with a general physical examination. Methods of restraint, illumination and magnification required and different signs of ocular diseases have been described. The gross examination on conformation and symmetry of the eyes and its adnexa, global size and position reveals signs of ocular discomfort which include blepharospasm, discharge, ptosis, swelling or muscle atrophy. Both peripheral and central vision can be assessed by performing a menace test and this will evaluate the cranial nerves II and VII. The palpebral reflex can be evaluated to test the function of sensory innervations of eye. The evaluation of pupillary reflex was also described.

2.4.2. Examination of Ocular Structures

According to Miller and Crenshaw (1988) direct diffuse illumination can be used to assess corneal contour, clarity, symmetry and contact between the eyelid margins and cornea.

Sansom (2000) reported that in diseases of anterior chamber, an early examination was necessary before the corneal endothelium becomes compromised, preventing a thorough examination as a result of corneal oedema.

As per Felchle and Urbanz (2001) the cornea must be examined under adequate illumination and magnification and any deviation from the normal transparent avascular state of the cornea indicates a disease process.

In a retrospective study on corneal ulcers in horses Michau *et al.* (2003) reported employing techniques like fluorescein staining, slit- lamp biomicroscopy and direct ophthalmoscopy. Additional diagnostics included cytology, aerobic culture and sensitivity, fungal culture and histopathology.

2.4.3. Special diagnostic procedures

2.4.3.1. Fluorescein Staining

Slatter (1973) reported that fluorescein does not enter the cells but it enters the defects in the corneal epithelium and stains the tissue fluid surrounding the epithelial cells and in the corneal stroma. The use of a fluorescein- rose bengal mixture, followed in some cases by alcian blue solution in evaluating the conjunctiva and cornea of the dog was studied which revealed that the mixture of fluorescein and rose bengal as a more convenient method of administering the dyes than the use of each alone. The use of fluorescein either as a sterile solution or impregnated paper strips was also recommended.

Bedford (1987) reported the use of fluorescein in demonstrating the loss of corneal epithelium and to identify the presence of a corneal ulcer or erosion.

Miller (1988) suggested that fluorescein dye strips were useful for determining corneal integrity and patency of the nasolacrimal ducts. Sterile wash solutions were then used to flush out excess stain.

Helper (1989) described the use of fluorescein as a non-toxic ophthalmic stain to bring an abrasion or ulceration into view. It was opined that use of dye impregnated strips eliminated the chance of introducing infections as seen with solutions.

Gelatt (1991) recommended the use of fluorescein impregnated paper strips rather than solutions. It was explained that this hydrophilic substance will not penetrate the intact corneal epithelium which has higher lipid content. The dye gains access to the hydrophilic stroma when the epithelium becomes damaged and it can be visualized as a bright green fluorescence.

According to Felchle and Urbanz (2001) fluorescein was useful for detecting epithelial defects and it is available as impregnated strip or as a 0.5% to 2% solution. It was opined that solutions can become easily infected and so the paper strips were best suited for clinical use and that it can also be used for detecting aqueous humor leakage through a corneal deficit.

2.4.3.2. Corneal Microbial Culture

Miller and Crenshaw (1988) pointed out that for a microbial culture the material should be obtained before adding a topical anaesthetic, because they may contain preservatives and antimicrobial additives that will inhibit microbial growth in culture.

As per Gelatt (1991) corneal culture and sensitivity was a useful tool in identifying specific causes of external ocular infections and to choose the most effective topical antibiotic.

Wilkie and Whittaker (1997) suggested that culture and sensitivity and cytology should be considered for all corneal ulcers and perforations.

Massa *et al.* (1999) used sterile swabs to collect material from the center and periphery of the epithelial defects in different species of animals and birds and used for diagnosis of infectious ulcerative keratitis. During the study *Streptococcus* spp and *Staphylococcus* spp, were found to the most prevalent bacterial isolates.

Felchle and Urbanz (2001) opined that culture and sensitivity testing for corneal or conjunctival disease provides useful information for establishing a diagnosis and determining the appropriate antimicrobial therapy. Culture swabs moistened with sterile saline may be used to obtain the samples.

2.4.3.3. Ocular Exfoliative Cytology

Lavach *et al.* (1977) obtained conjunctival scrapings from both normal and infected eyes. Keratinized epithelial cells and leucocytes were uncommon in normal eyes. In inflamed conjunctiva the initial inflammatory cell response was seen specific according to the cause. Leucocytes were the predominant cell type in all cases of acute and chronic bacterial conjunctivitis.

Corneoconjunctival cytology was recommended to demonstrate the intensity of inflammation and the presence of infection by the type of inflammatory cells (Gelatt, 1991). It was also explained that the smears or scrapings obtained from cornea, conjunctiva, nictitating membrane and eyelid aid in diagnosis.

Massa *et al.* (1999) used corneal scrapings from the lesions in different species of animals and birds and compared its usefulness with microbial culture. It was found out that each one has significant use in ophthalmic treatment.

Felchle and Urbanz (2001) described methods to obtain samples for conjunctival and corneal cytology using cotton or Dacron swab, a cryobrush, a malleable platinum spatula or the blunt end of a scalpel blade and also the procedure for preparing, staining and examining the smears.

2.5 CORNEAL PIGMENTATION

According to Roberts (1954) initial changes of pigmentation were observed on the corneal side of the limbus and vascularization accompanied

pigment deposit. Pigmentation may be patchy or covering the entire cornea which is associated with severe signs of local inflammation. He found out that the condition was more common in breeds with large, prominent eyes and those with dark conjunctival pigmentation were prone to develop corneal pigment under certain stresses. It was also described that practically all the pigment in the corneas was melanin. It was suggested that germinal epithelium, rather than the limbal pigment produces corneal pigmentation.

Bellhorn and Henkind (1966) conducted microscopic examination of pigmented canine corneas and found that glaucoma, eyelid abnormalities, exposure keratitis and 'pannus' in the German Shepherd Dog were some predisposing conditions which may result in a pigmented cornea. The study emphasized that superficial corneal pigmentation was derived from the invasion of limbal melanocytes, which was preceded by corneal vascularization.

As described by Wilcock (1993), corneal pigmentation often accompanies chronic corneal irritation. There was usually evidence of chronic corneal stimulation including vascularization.

According to Grevan (2000) corneal pigmentation was most common in brachycephalic breeds of dogs. It was suggested that most of them will be benefited from medial canthoplasty eliminating corneal exposure.

Whitley (1991) described that pigment at the limbus was scattered in all layers except the superficial squamous cells. Pigment was produced in the corneal epithelium and subepithelial stroma. The most common cause was chronic irritation due to various causes and treatment includes correction of the inciting cause and preventing the progress of the pigmentation by methods like scarification to remove the blood vessels, beta ray application, topical application of corticosteroids *etc.*

2.6 CORNEAL REPAIR

According to Krawitz (1963), the immediate repair of corneal wounds took place by cell migration, which quickly provides a new protective cover. At the same time restoration of tissue substance was by cell division which took several days to complete.

Wilcock (1993) described that corneal stroma consists of fibrous tissue lamellae in between which the fixed cells or keratocytes were present. They can transform into fibroblasts when deep corneal injury occurs and form scar tissue. It was opined that healing of the epithelial surface of cornea took place in two stages *viz.*, cell migration to cover injured area and mitosis to reconstitute the normal number of epithelial cells. Healing of small epithelial defects took place without cell multiplication, but simply by cell migration in the first 24 hours.

Fibroblastic changes in the corneal stroma, following experimental corneal grafting in dogs were observed by Chandrashekar *et al.*(1997b). It was reported that the proliferated fibrous tissue was seen projecting into the anterior chamber through the gap existed between descemet's membrane.

2.7. HEALING OF STROMAL DEFECTS

Wilcock (1993) described that those defects involving the epithelium alone or epithelium and superficial stroma heal by epithelial sliding followed by mitosis and described rapid healing of shallow, uninfected corneal ulcers. It was also reported that epithelial cells creating an epithelial facet, which was permanent but clinically insignificant, filled shallow defects in superficial stroma. According to this, deeper defects including more than the outer third of stroma must heal by epithelial sliding and replication combined with stromal metaplasia. In deeper defects complete restoration of normal stroma never occurs and a residual scar may remain. The steps in healing of corneal

perforations were described. Fibrin deposition and epithelial sliding in large gaping wound, which becomes filled with proliferating epithelium and stromal fibrous tissue, may protrude into the anterior chamber.

Healing of induced corneal ulcers in horses was studied by Neaderland *et al.*, (1987) in which ulcers healed in an average period of 11days in treated and 13.5 days in non-treated group.

Befanis *et al.* (1981) studied the endothelial repair of the canine cornea and described that it involved both mitosis and migration of the healthy cells. The time required for total re-endothelialization to occur corresponded to the time needed for complete resolution of corneal oedema.

According to Whitley (1991) in the first 24 hours of wounding healing was characterized by proliferation and migration of polymorphs and peripheral keratocytes. In a perforated wound collagen fibers were first replaced by fibrin matrix. Collagen then accumulated very rapidly during the first 2 weeks of healing and more slowly thereafter due to reduced rate of collagen synthesis and/ or increased breakdown. Proteoglycans were also synthesized during healing. According to him the endothelial repair was slower which enlarge and migrate to maintain a functional monolayer. Whenever this was not accomplished, a focal area of corneal oedema was observed.

As described by Malenda (2000) healing of corneal ulcer takes place by a combination of mitosis and cellular migration and it usually leaves a central depression in the cornea known as a corneal facet. An uncomplicated ulcer will be exacerbated by chronic irritation and conformational abnormalities. According to him pathogenic organisms invade the canine cornea only in the presence of an ulceration, which may be caused by a variety of reasons.

In the studies conducted by Michau *et al.* (2003) the duration of clinical signs prior to referral was not associated with age, breed, sex, treatment received at initial examination or time to re-epithelialization.

2.7.1. Corneal Response to Injury

Wilcock (1993) opined that acutely injured cornea cannot respond with acute inflammation because it lacks blood vessels. Oedema as the hallmark of corneal injury results from injury to the corneal epithelium or endothelium. It was described that with chronic corneal disease the cornea may undergo metaplasia resembling the limbic sclera and thus requires a full range of inflammatory responses available to vascularized tissue. As described by him, neutrophils reach the wound within a few hours, via the tear film and attracted by proteases released by the injured epithelium, which limit chances of infection and aid in healing.

2.7.2. Causes of Corneal Injury

Wilcock (1993) explained that corneal injury may result from physical or chemical trauma, microbial agents increased intra- ocular pressure and rarely from inborn errors of metabolism.

Wilkie and Whittaker (1997) reported that corneal perforations can occur as a result of a complicated corneal ulcer that progress to a descemetocoele and subsequently ruptures or may occur acutely as the result of trauma.

In horses, the superficial ulceration was described to involve traumatic events as an inciting cause or in association with it (Michau *et al.*, 2003).

2.7.3. Corneal Vascularization

Krawitz (1963) opined that the corneal vascularization may be due to a nutritional inadequacy during wound healing. It was stated that vascularization occurs when there is vitamin or amino acid deficiency. Direct vascularization results in augmented nutrition of the cells of the cornea aiding in healing of the tissue.

Ulceration of the cornea may result in excessive vascularization even though satisfactory healing has been obtained or in the formation of exuberant granulation tissue on the corneal surface (Startup, 1984).

Culton *et al.* (1990) investigated the effect of oxygen on corneal neovascularization process. It was stated that tissue hypoxia plays an essential role in the induction of neovascularization and also leads to increased capillary density in skeletal and cardiac muscle. Rat eyes, undergone chemical cautery, were selected and provided goggles to limit the oxygen availability at the corneal surface. All the corneas manifested prominent corneal vascularization at oxygen saturations less than 100%. The results were comparable to earlier studies, which showed that hypoxia stimulated new vessel formation.

As described by Michau *et al.* (2003) non-healing corneal ulcers in horses were associated with superficial or deep neovascularization. It was found that the presence of corneal vascularization was not associated with age, breed, sex, duration of ulceration prior to referral or time to re-epithelialization.

2.7.4. Corneal Oedema

According to Krawitz (1963), general opaqueness of the cornea will follow serious interferences with corneal metabolism, which results from denaturation of tissue proteins. It was opined that the opacities following

wound healing were most often irreversible due to deposition of collagen fibrils not characteristic of the cornea.

Bedford (1987) described that, deep ulceration was usually accompanied by marked corneal oedema and there may be stromal vascularization.

Wilcock (1993) opined that oedema occurs rapidly following injury and results from inhibition of lacrimal water flow through the damaged corneal epithelium or failure of extrusion of electrolytes (and thus water) by the corneal endothelium and such oedematous cornea appear clinically opaque. Corneal vascularization in response to severe injury was accompanied by oedema as fluid leaks from new capillaries into the interstitial spaces.

As opined by Sansom (2000) diseases involving the anterior chamber frequently damage the corneal endothelium, resulting in corneal oedema. It may also occur with contusion injuries to the head resulting in corneal oedema and intraocular haemorrhage. In penetrating injury, the wound results in a shallow anterior member and will be invariably accompanied by uveitis.

2.8. SIMPLE CORNEAL ULCERS

Startup (1984) reported that corneal ulceration was always accompanied by corneal oedema, vascularization, pain and photophobia. Other signs included, loss of transparency due to leucocytic infiltration or oedema. Ulceration may follow any condition in which the surface was not kept properly moistened and protected by the eyelids. Such ulcerations may be associated with disorders such as ectropion, exophthalmos, traumatic deficiencies in lid margins or any inability to close the eyelids. Due to this those breeds with protruding eye are particularly susceptible to corneal drying, particularly in the central areas where adequate spreading of the precorneal tear film may not occur. Cleanliness of the cornea and conjunctival sac has to

be ensured for healing to take place, which may be best accomplished by the use of saline irrigations and sponges. The necrotic tissue that may be present also required removal by debridement. In a few cases, de-epithelialization may be indicated. Paracentesis of the anterior chamber, by means of an incision at the limbus to release aqueous humor, was indicated in certain cases where the ulceration was deep with threatened perforation, or where there was a keratocoele or increased intraocular tension. This reduced the intraocular pressure, thus reducing the risk of perforation and increased the antibody and antibiotic content of the interior of the eye. Migrating columnar epithelial cells and subsequent proliferation to restore the thickness of the epithelium over a few days accomplished healing of large defects.

Bedford (1987) opined that though primary corneal ulcers were unusual, the exposed corneal stroma become very susceptible to secondary infection with *Staphylococci, Streptococci, Escherichia coli, Pseudomonas etc.*

Treatment of advanced corneal ulcers by using a pedicle graft from the bulbar conjunctiva was described by Hakanson and Merideth (1987). It was found to be effective in filling of stromal defects and effective against keratomalacia. The method required five to eight weeks of graft retention and prolonged medical therapy.

Wilcock (1993) reported that keratitis was caused by physical and chemical trauma, desiccation, bacterial or viral infections and rarely from primary degeneration of the corneal epithelium itself. Loss of epithelium initiates corneal reactions caused by tear imbibition, local production of cytokines and opportunistic microbial contamination of the wound. The leucocytes protect against pathogen and produce collagenases, proteases and cytokines and thereby may contribute to its progression. It was opined that, repair may fail in cases with microbial contamination and where the cause of

initial ulceration has not been corrected. Proteases and collagenases of microbial, leucocytic or corneal origin progressively liquefy the corneal stroma. Inflammation may involve the cornea, conjunctiva and uvea. The ulcer may heal by itself with vascularization and scarring proportionate to the severity of initial lesion.

Pickett (1995) reported the use of crosshatch keratotomy for persistent corneal erosions in dogs. It was opined that debridement of the loose epithelium and ulcer bed removed the abnormal epithelium and part of the abnormal basement membrane. Third eyelid flaps, soft contact lenses and collagen shields were used to provide support for the healing cornea and apply pressure to the migrating epithelium to improve its adhesion to the underlying corneal stroma. Results showed that crosshatch keratotomy enhanced healing of persistent corneal erosions, whether the technique was used alone or with a soft contact lens.

Suresh (1996) performed superficial keratectomy for the management of corneal wounds in canines and suggested it to be an effective method of treatment for such conditions.

Michau *et al.* (2003) studied on superficial non-healing corneal ulcers in the horse and assessed the effect of age, breed, sex, inciting cause of the ulcer and treatment on healing time of these ulcers. There was no significant difference in age, breed or gender when compared to similar cases reported contemporarily. The inciting cause was mostly associated with traumatic event. Involvement of right and left eyes was also found to be almost equal. The duration of clinical signs prior to referral was not associated with age, breed, sex, treatment received at initial examination or time to re-epithlialization.

2.9. ULCERATIVE KERATITIS

According to Whitley (1991) corneal ulcers were frequently traumatic in origin, but rapidly contaminated by bacteria. Diagnosis could be done based on cytological examination and on culture and sensitivity testing of corneal samples. It was suggested that progressive deep ulcers were potentially vision and globe threatening and so the therapy must be aggressive. Infected or severely traumatized ulcer margins were to be debrided. In large descemetocoeles, an allograft of the cornea into the defect hastened healing.

Various corneal disease conditions including ulceration were described by Crispin (1993) along with the etiological factors and treatment modalities.

In the studies conducted by Massa *et al.* (1999) an infectious ulcerative keratitis was suspected when a corneal epithelial defect was accompanied by stromal loss, mucopurulent discharge and intrastromal cellular infiltrate with or without corneal vascularization.

Malenda (2000) explained that loss of corneal epithelium results in infection with bacteria from the conjunctival sac. It leads to deepening of the ulcer and may result in perforation, if goes untreated.

In a case report of ulcerative keratitis Landry *et al.* (2004) observed clinical signs like miosis, blepharosapsm, epiphora and photophobia. The inciting causes included trauma, keratoconjunctivitis sicca, exposure keratitis, foreign body, distichiasis, entropion, trichiasis, ectopic cilia and exposure to caustic substances. Diagnosis was based on the results of culture, corneal cytology and histopathology and on response to treatment.

2.10. STAPHYLOMA

Bedford (1987) reported penetrating wounds of cornea and subsequent staphyloma formation provided potential tract along which infection enters the

eye and so all such wounds should be repaired as soon as possible to reduce the risk of both. Topical and systemic broad-spectrum antibiotics together with cycloplegic and mydriatic therapy were essential.

Brightman *et al.*(1989) reported the treatment of autogenous lamellar corneal grafting in dogs with chronic descemetocoeles and other perforating corneal diseases. It was reported that this technique resulted in rapid resorption of severe corneal diseases, with a high success rate.

As described by Wilcock (1993) in corneal perforations the iris flows forward to plug the defect and subsequently become incorporated into the corneal scarring and the condition leads to permanent anterior synechia.

Wilkie and Whittaker (1997) described the occurrence and treatment of iris prolapse. Prolapsed iris may be replaced or excised under general anaesthesia.

Glover (2000) reported that the eye with perforating corneal ulcer would be usually presented with a formed anterior chamber because the defect was plugged with fibrin and an anteriorly displaced ulcer.

According to Sansom (2000) iris prolapse may occur through a penetrating corneal wound. In such cases, distortion of the pupil towards the corneal wound was a good indicator of iris prolapse. As therapy, surgical repair of the wound and replacement of the iris was reported to be effective.

2.11. PROPTOSIS OF THE GLOBE

As described by Bedford (1987) proptosis occurs due to orbital trauma or acute orbital cellulites and it may result in exposure keratitis or ulceration if neglected. It was described that the condition occurs commonly in brachycephalic breeds owing to their shallow orbit and wide palpebral fissure. Recommended treatment consisted of canthotomy and temporary

tarsorrhaphy, which should be kept for 10 to 14 days. The potential corneal damage and desiccation also complicate the proptosis.

Kern (1991) described the clinical signs, conformational predisposition and treatment of traumatic proptosis in dogs. Factors like brachycephaly, shallow orbit, blunt trauma or traction applied to the orbit, globe or facial skin *etc,* were found to predispose to proptosis. Lateral canthotomy and gentle pressure were recommended for correction followed by tarsorrhaphy, which has to be retained for 7- 10 days.

Gilger *et al.* (1995) studied the occurrence and treatment of traumatic ocular proptoses in dogs and cats. They reported that the prognosis for having a viable, visual eye after surgical replacement of a proptosis was poor. Sequelae in dogs included lagophthalmos, strabismus, keratoconjunctivitis sicca, keratitis, uveitis, lens luxation, cataract, glaucoma, retinal detachment, retinal degeneration, optic nerve degeneration, phthisis bulbi and blindness. Lateral canthotomy and tarsorrhaphy were performed in proptosed eyes followed by atropine, corticosteroids and antibiotics. A major finding was that proptosed eyes of brachycephalic dogs had a better chance at a visual outcome than eyes of nonbrachycephalic dogs.

Glover (2000) reported that prognosis for vision in a prolapsed globe was poor in dogs. It may be unilateral or bilateral and was invariably secondary to trauma.

2.12. CORNEAL INJURY/ LACERATIONS

According to Bedford (1987) corneal penetrating wounds and burns require emergency attention. Potential infection and subsequent uveitis were to be considered and both topical and systemic antibiotics together with mydriatric and cycioplegic therapy must be instituted.

Whitley (1991) described surgical intervention of traumatic injuries. It was recommended that corneal lacerations, deeper than one half the thickness of the cornea with edges that gape and full thickness lacerations were to be repaired surgically. Prognosis of the condition depends on the initial depth and severity of the wound.

Glover (2000) reported that deep and uncomplicated corneal lacerations could be sutured directly and topical antibiotics could be effectively used.

2.13. MICROBIAL FLORA OF CANINE EYE

Sansom (1988) reviewed the reports on ocular pathogens and reported that the normal flora was influenced by the geographical location, climate and seasonal factors, as well as there being a breed influence. According to him surveys had shown *Staphylococcus, Streptococcus, Corynebacterium, Neisseria, Pseudomonas* and *Moraxella* species to be the most prevalent.

Gerding *et al.* (1993) reported the occurrence of different species of bacteria and fungi in the eyes of clinically normal dogs. It was found that gram positive organisms were the predominant conjunctival bacteria, including *Streptococcus* spp. being the most widely isolated species. Other isolates included *Corynebacterium* spp. and *Streptococcus* spp., *Escherichia, Neisseria, Pseudomonas* and *Klebsiella* comprised the gram negative genera isolated. It was described that bacterial and fungal microorganisms were isolated either singly or concurrently from the dogs. The data indicated *Staphylococcus* spp. as the most frequently isolated conjunctival and eyelid organism, indicating a role of endogenous *Staphylococcus* spp. as potential pathogens.

2.14. OCULAR THERAPEUTICS

The corneal epithelial healing rate was studied by Martin (1971) in dogs treated with topical antibiotic, mineral oil-antibiotic, vitamin A-antibiotic, subconjunctival repositol corticosteroid and vitamin A-corticosteroid and in controls. The mean healing rate of corticosteroid group was significantly increased over the control group. In this group there was a mean retardation in healing and the higher risk of corneal perforation. The vitamin A preparation reversed the inhibition of healing in the antibiotic treated group. A deficiency of vitamin A resulted in inhibition of corneal wound healing by decreasing mitosis, but vitamin A in excess has not increased normal wound healing.

Startup (1984) reported that fat soluble drugs penetrate the corneal epithelium, whereas water- soluble drugs penetrate, the entire cornea. Drugs must, therefore, be both fat and water soluble in order to penetrate the entire cornea. For initial treatment of corneal ulcers, any broad- spectrum antibiotic, effective over a wide range, including *Pseudomonas* spp. might be used. Topical application of steroids was recommended for the control of unwanted vascularization once the healing process was complete and the cornea becomes fluorescein- negative.

Neaderland *et al.* (1987) used 1% atropine and chloramphenicol ophthalmic ointments after experimental induction of corneal ulcers in horses.

Commonly used ocular antibacterials and antibiotics were reviewed by Sansom (1988) and reported the need of systemic administration of drugs in case of severeinfections. It was reported that chloramphenicol ointment gave a better corneal penetration than repeated drop administration. He also described that aminoglycosides, penicillins, polypeptide antibiotics, tetracyclines, sulphonamides, cephalosporins *etc.*, were effective against different groups of microorganisms.

Helper (1989) explained the use of mydriatic and cycloplegic agents, which were useful to dilate the pupil, prevent adhesions and to relieve pain by paralyzing the ciliary body. Topical and systemic use of antibiotics and non-steroidal anti-inflammatory agents were also recommended.

During the study involving corneal grafting in dogs, Chandrashekar *et al.* (1997a) used bandage, plastic eye shields and modified head shield to prevent auto mutilation. They used Gentamicin topically and systemically along with triamcinolone sub- conjunctivally on tenth post-operative day.

Wilkie and Whittaker (1997) described the post- operative therapy of staphyloma including topical and systemic broad spectrum antibiotics, topical mydriatic agents, an Elizabethan collar and exercise restriction for 10 to 14 days.

Moore (2001) described about diagnostic agents, *viz.,* nonsteroidal anti-inflammatories, antimicrobial agents and mydriatic/ cycloplegic agents. The NSAIDs were described to be indicated for the treatment of uveitis, blepharitis, conjunctivitis or keratitis. Among them flurbiprofen 0.03 % was reported to prevent intraoperative miosis and as effective in stabilizing blood aqueous barrier in canines. In case of ulcerative keratitis loss of corneal epithelial barrier develops and drug penetration becomes possible. Systemic therapy was indicated for deep intraocular or orbital infections.

Munro (2001) described about various forms therapeutic agent used in ophthalmology. It was opined that topical therapy was the route of choice for the majority of ocular surface conditions, with the eyelids, conjunctiva, cornea and in few instances iris being the target tissues. Systemic therapy was, in general, inappropriate for corneal disease, due to the absence of vessels in the normal cornea. Corneal ulcerations were to be treated with topical antibiotics until complete epithelialization. Topical non-steroidal anti- inflammatory drugs with good corneal penetrating ability like flurbiprofen sodium and

diclofenac sodium were recommended over topical steroids especially in cases with penetrating injuries where intraocular infection was thought likely. Cytology and bacterial culture were reported to be necessary in case of refractory ulcers.

Hendrix *et al.* (2001) conducted experiments to evaluate the effects of antibiotics on morphologic characteristics and migration of canine corneal epithelial cells in vitro. It was found that chloramphenicol and tobramycin caused the least effect on cellular morphologic characteristics. It was also described that *in vivo* all antibiotics were diluted immediately after application to the eye, by the basal tear volume, reflex tearing caused by irritation and normal physiologic drainage.

Michau *et al.* (2003) used topical medications including prophylactic antimicrobials (until the epithelialization was complete), antifungals, mydriatic-cycloplegic agents and oral anti- inflammatories for the treatment of corneal ulcers in horses.

2.15. COLLAGEN IN CORNEAL HEALING

2.15.1 Collagen in Cornea

According to Helper (1989) cornea consists of a large amount of collagen, one of the factors involved in wound healing. The corneal stroma comprising about 90% of the corneal thickness was constituted mainly by collagen fibrils, which play an important role in maintaining corneal transparency.

Whitley (1991) described that the collagen fibrils within a lamella were all parallel, but between lamellae they vary greatly in direction. The bulk of corneal stroma was composed of thin collagen fibrils that traverse the full diameter of cornea which was found to be similar to the collagen from other

sources. Physical rearrangement of the precise collagen organization resulted in opacity of the cornea.

2.15.2. Collagen as a Drug Delivery System

Gelatt (1991) described the use of collagen inserts for drug delivery. Its use reduced the frequency of drug administration which was suitable in chronic eye diseases. It was opined that when the collagen was exposed to aqueous media, hydrolytic changes take place leading to the release of the drug. In another study he found complete dissolution in eight hours. He had also mentioned about the use of collagen shield made from porcine collagen which was useful in treating various ocular surface disorders. It was reported that its effect was more pronounced during the first eight hours of therapy.

Moore (2001) described about collagen shields as dissolvable/ erodable devices used in the form of ocular inserts. They were placed on the cornea or in the conjunctival cul- de- sac to allow constant release of medications onto ocular surface.

Hariprasad *et al.* (2004) described that the corneal collagen shield was originally developed as a bandage lens for the treatment of corneal epithelial damage and investigations have demonstrated that the collagen shield may be well suited for drug delivery in the perioperative setting and has several advantages over topical and subconjunctival routes of antibiotic administration. Studies were conducted to determine the intraocular penetration of moxifloxacin 0.5% using a 24-hour dissolvable cross-linked corneal collagen shield device. Topically applied antibiotics have been used in conjunction with the shield to promote healing of corneal ulcers. Collagen shields were fabricated with foetal calf skin tissue and originally developed as a corneal bandage. These devices, once softened by the tear fluid, form a thin pliable film that confirms exactly to the corneal surface, and undergoes

dissolution up to 10, 24 or 72 hours. Collagen film proved as a promising carrier for ophthalmic drug delivery system because of its biological inertness, structural stability and good biocompatibility.

2.15.3. Role of Collagen in Corneal Healing

Role of collagen as a biomaterial was reported by Simpson (1983). He opined that it was less antigenic and the control of solubility, strength and resorption could be accomplished through change of composition and structure and these factors makes collagen an ideal material for use as a tissue substitute. He suggested gamma radiation from a cobalt-60 source for sterilization of these products for medical use with slight denaturation. The ophthalmic use of collagen was also described which included sutures, purified collagen film for keratoplasty, collagen tape for scleral buckling and in vitreous replacement.

Shaker *et al.* (1989) investigated the effect of collagen bandage lenses on corneal epithelial wound healing after mechanical debridement of the cat cornea. Non- cross- linked collagen shield made from porcine scleral tissue and as such consisted primarily of type I collagen and sterilised by gamma irradiation were used in the treated group. The mean expected healing time for the control group was 49.32$\pm$ 10.22 hour and for the treated group was 37.97$\pm$ 4.07 hour using the individual regression analysis. Shield dissolution appeared to involve both enzymatic and mechanical action. The rate of shield dissolution was thought to be depending on several host factors, including tear volume, tear enzyme concentration, degree of inflammation and blink rate. On analysis of the data the effect was found to be most pronounced during the first 8 hour after debridement. One major advantage over the hydrophilic lenses was higher oxygen permeability through collagen which also increases as collagen biodegrades.

Robin *et al.* (1990) studied the effect of collagen shields on rabbit corneal reepithelialization after chemical debridement. They used porcine collagen shields in which the collagen was cross-linked to the degree that dissolution in vitro occurs in approximately 72 hours. It was found that the healing rate was directly related to the initial wound area, with larger wounds demonstrating a faster initial decrease in wound areas. They concluded that when used in patients with corneal epithelial defects, collagen shields were generally well tolerated and did not appear to interfere with the reepithelialization process.

The oxygen permeability of collagen shields was measured by Weissman *et al.* (1990). It was opined that, as the corneal epithelium is dependent on atmospheric oxygen reaching the corneal surface through the tears, the increased oxygen transmissibility of a contact lens may lead to improved epithelial healing. It was found that the measured values were similar to that expected for a hydrogel material and that the water content of both hydrogel and collagen contact lenses defines the permeability of these materials.

Geasey *et al.* (1992) reported that the collagen shields were proved to be highly oxygen permeable, promote epithelial healing, decrease inflammatory cell infiltration and reduce stromal oedema. They conducted experiments in human subjects to investigate the use of collagen shields as a vehicle for collecting and studying migratory cells on human corneas. They placed 24-hour cross-linked collagen shields in patients who underwent penetrating keratoplasty, those who underwent cataract surgery and normal volunteers as bandage lenses. The shields were removed after 24 hour after application and processed for microscopic examination and for histochemical studies. It was revealed that large number of polymorphonuclear leucocytes was adhering to the shield. He reported that the irregularity of the shield edge and signs of cellular disintegration indicated the dissolution of the shield not

only by tear enzymes and the mechanical action of the eyelid but also by the cellular phagocytic activity. The changes of the shield was consistent with the clinical observations that the dissolution rate was generally higher in postoperative eyes with increased inflammation cell counts compared with that of the normal volunteers.

Barros (1999) reported corneal repair using synthetic or biological materials and studied the clinical and histological aspects of the experimental use of preserved equine pericardium in the repair of superficial defects of the cornea of the dog. The epithelialization began at day 7 and was complete at day 30. The opacification at the graft site could be minimized when treated by third eyelid flap, contact lens and temporary tarsorrhaphy.

Full thickness corneoscleral defects in a German Shepherd Dog was repaired using porcine small intestinal submucosa (SIS) by Lewin (1999). The material was described to be acellular and composed mainly of extracellular connective tissue. It was also reported the use of SIS as a vascular graft, for the repair of bladder wall and Achilles tendon *etc.*, where regeneration of the recipient organ resulted. In no case immune rejection of the xenograft observed since it was acellular collagen which was the same between species.

Orwin and Hubel (2000) investigated the effectiveness of a tissue-engineered collagen sponge as a substrate for the culture of human corneal cells. Human keratocytes, epithelial and endothelial cells were cultured separately on collagen sponges composed of native fibrillar collagen. Reviewed studies had shown that collagen can support epithelial cell migration and differentiation. Collagen sponges which were prepared from Bovine Type I dermal collagen with pores for cellular infiltration. The results indicated that collagen sponge promoted epithelialization, keratocyte infiltration and attachment and normal morphology of endothelial cells. Other

studies also showed that the fraction of light transmitted through a collagen sponge cultured with keratocytes was between 50% and 60% of that observed with native excised rabbit cornea. These results indicated that the collagen sponge may have the potential to promote wound healing in the eye.

Willoughby *et al.* (2002) opined that the biocompatibility and biodegradability of collagen, along with its ability to support corneal epithelial cells in culture, led to the development of collagen corneal shields as an ocular surface bandage. Collagen shields are currently manufactured from porcine scleral tissue or bovine corium (dermis) collagen and contain mainly type I collagen and some type III collagen. Variations in collagen cross- linking induced with ultraviolet light (UV) during manufacture dictate lens duration before dissolution. Animal models with corneal epithelial defects and superficial keratectomy and radial keratotomy wounds have demonstrated that collagen shields can enhance re-epithelialization and reduce stromal inflammation and oedema. For acute epithelial defects collagen shields represented a good alternative to conventional methods to promote re-epithelialization. Studies have shown that shields with a dissolution time of 72 hours might be more effective. They were found to be enhancing re-epithelialization and also reducing any possible keratocyte loss and potentially reducing subepithelial haze. In microbial keratitis, collagen shields can contribute to healing by enhancing drug delivery, promoting epithelial and stromal healing neutralizing collagenases generated from leucocytes and bacteria and reducing corneal inflammation and oedema. A presoaked collagen shield with less intensive supplemental topical therapy was suggested, as an acceptable alternative to intensive, fortified topical treatment in microbial keratitis.

Bussieres *et al.* (2004) reported the use of autologous, homologous, xenologous and biomaterial grafts for repair of lamellar keratoplasty. In a retrospective study conducted by him on the use of porcine small intestinal

submucosa for the repair of full thickness corneal defects in dogs, cats and horses it was shown that collagen based material had the advantage of being cost effective, commercially available and easy to handle. It is also easy to be stored and could be handled aseptically as the product was sterile and free of pathogens.

Barros *et al.* (2005) used amniotic membrane in the reconstructive surgery of cornea in dogs and cats with good results. According to them, the amniotic membrane acts as a substrate for epithelial growth as well for axonal regeneration, and is a suitable way of epithelialization by facilitation of migration of the epithelial cells, reinforcement of the adhesion of basal epithelial cell, promotion of epithelial differentiation and through its role in preventing apoptosis. It was also said to have anti- scarring effect as a result of the anti- inflammatory mechanisms and prevention of fibroblastic activation. An anti- microbial effect has also been demonstrated against a wide variety of bacteria.

Materials and Methods

3. MATERIALS AND METHODS

The study was conducted in eight dogs of different age, breed and sex presented to the Veterinary Hospitals of College of Veterinary and Animal Sciences at Mannuthy and Kokkalai.

3.1. SELECTION OF CASES

All the animals presented with a history of eye affections were thoroughly examined and eight dogs with corneal affections were selected for the study. One animal (Pug) with bilateral involvement of the cornea was considered as two separate cases (Case Nos. VII and VIII). Thus nine cases of corneal lesions were utilized for the study. In all the selected cases, the eye was thoroughly cleaned and ciprofloxacin[1] antibiotic eye drops was instilled. Since the animals were under medication till the date of presentation, surgical intervention was carried out on the day of presentation itself in all cases except Case I, with a fresh corneal injury.

3.2. OUTLINE OF WORK

The affected eyes were thoroughly examined and those cases with ulceration / laceration/ penetrating wound and proptosis with corneal injury were identified and selected for the study. The cases were numbered as I, II, III, IV, V, VI, VII, VIII and IX. Sterile swabs for culture and sensitivity from the corneal lesions were taken prior to any medication. The affected eyes were thoroughly cleaned with sterile normal saline. After scarification, debridement or suturing, the collagen diskette was placed over the defect on the cornea and tarsorrhaphy was performed to retain the diskette in position. Postoperatively ophthalmic antibiotic drops, according to antibiotic sensitivity test, were instilled 4 times

[1] Ciplox eye/ear drops(0.3%), Cipla Ltd,Verna, Goa. 5ml bottle.

daily. The eye was examined on 3rd, 7th, 14th, 28th and at 60 days and the observations were recorded.

3.3. CLINICAL EXAMINATION

3.3.1. History

The signalment, anamnesis, symptoms noticed by the owner, duration of illness and details of former treatments were recorded. The breeds of animals subjected to the study included, German Shepherd Dog (1), Pug (5), Spitz (1) and Labrador Retriever (1). Out of eight animals four were males and the rest were females.

3.3.2.Clinical observations (Table 2)

All the animals selected for the study were examined for their general condition, clinical condition of the eye including nature and extend of lesion, nature of ocular discharge and vascularization if present, and the previous medications given were recorded. The duration of the illness at the time of presentation for treatment varied from one day to two weeks.

3.3.3. Collection of materials

Sterile swabs for culture and sensitivity and lacrimal smear for exfoliative cytology were taken and the results were analyzed. Blood samples were collected in EDTA pre-operatively for haematological evaluation *viz.*, haemoglobin concentration, volume of packed red cells(VPRC), total leucocyte count(TLC) and differential leucocyte count (DLC). Wet film examination for blood parasites and fluorescein dye test were also carried out at the initial stage of examination.

3.3.4. Ophthalmic cases selected

The ophthalmic conditions selected for the study included deep corneal injury (1), corneal ulcer (4), ulceration with descematocoele (1), staphyloma (2) and corneal injury with proptosis (1). (Table 1)

3.4. TREATMENT ADOPTED

3.4.1. Collagen diskette

Collagen diskettes prepared from bovine Achilles tendon were used for the present study (Plate1). These were supplied by the Central Leather Research Institute (CLRI), Adyar. During its preparation the tendon is chopped and subjected to enzymatic processes that make it a suitable material for protection and promotion of corneal wound healing. It appears as a semiluscent sheet when dry. The material was moistened with sterile phosphate buffered saline or any antibiotic solution to make it pliable and was kept over the corneal lesion (Plate 2). It was supposed that the collagen will then be subjected to natural enzymatic processes in the eye and the antibiotic will be released over a prolonged period of time (Moore, 1995). The material will be absorbed completely within 72 hours according to the manufacturers.

3.4.2. Preparation of the eye

All cases of corneal ulceration and laceration were referred by the local veterinarians and were under antibiotic treatment for a period of two days to two weeks except the cases with fresh injury (Case I). Affected eye was thoroughly irrigated with sterile normal saline, and cleaned with sterile cotton to remove the accumulated ocular discharge, dirt and tissue debris and ciprofloxacin eye drops were instilled.

3.4.3. Anaesthesia

All the dogs were maintained under general anaesthesia during surgery. Atropine sulphate[2] at a rate of 0.045 mg/kg body weight was administered as the pre-anaesthetic and after 15 minutes, Xylazine hydrochloride[3] was given intramuscularly at a rate of 2mg/kg body weight. Ketamine hydrochloride[4] at a rate of 5mg/kg body weight was given intramuscularly to induce general anaesthesia. Anaesthesia was maintained using a combination of equal volumes of xylazine and ketamine intravenously to effect.

On an average, 0.341 mg of Atropine sulphate, 30 mg of Xylazine hydrochloride and 75.3 mg of Ketamine hydrochloride were used for anaesthesia. On review of cases for subsequent observations, sedation with xylazine was used only in animals which were difficult to handle. In all the cases, topical anaesthetic of choice was Lignocaine hydrochloride[5] (4%).

3.4.4. Surgical procedure

3.4.4.1. Corneal injury

Two animals (Nos. I and IV), were affected with corneal injuries of different depth and characteristics.

Case I: The injury was fresh, deep and a reddish mass was projecting out from the corneal surface towards the ventro- medial aspect close to the centre of the cornea (Plate 3). The mass projected out was easily removed on manipulation, being a

---

[2] Atropine sulphate injection IP. (0.6mg/ml), Mount Mettur Pharmaceuticals Ltd., India
[3]Xylaxin, Indian Immunologicals Ltd., (20 mg/ml), Guntur dist., Andhra Pradesh, 2ml vial
[4] Ketmin 50, (50mg/ml), Themis Medicare Ltd., Mumbai, 2ml ampoule
[5] Xylocaine, (4%),Astra Zeneca Pharma India Ltd, Bangalore. 30 ml vial

friable mass of fibrin clot. On removal of the clot, the defect in the cornea was visible with a 2- 3 mm gap from which the descemet's membrane was slightly projected out (Plate 4). The edges were scarified and were sutured by 8/0 braided silk in simple interrupted, partial thickness pattern. The operation was carried out using ophthalmic operating microscope. Complete apposition was not achieved and sutures were removed after 2 weeks.

In Case IV, superficial corneal injury was present, which was caused subsequent to proptosis and exposure of cornea. The eye ball was remaining proptosed for the last two weeks prior to initial presentation of the case four days back. On that day, the eye was thoroughly irrigated with sterile saline and the debris was removed. The eye ball was reduced to normal position with constant gentle pressure and tarsorrhaphy was performed. On the day of surgery, the surface was only scarified to remove the dead tissue and debris.

3.4.4.2. Corneal ulceration

Cases II, III, VII, VIII and IX were presented with deep corneal ulceration. The duration of illness and previous medications were highly varying (Table 1).

Case II: The case was presented with a corneal ulceration which was being treated with topical chloramphenicol[6]. The defect, about 3mm diameter, was not healed and central cornea was found to be involved which was demonstrated with the fluorescein dye retention (Plate 5).

Case III: The case was presented with a very extensive mid- stromal ulceration (Plate 6). It was noticed one week back and was being treated with

[6] Chloromycetin Apli Caps (1%), Parke- Davis, Mumbai.

Betamethasone-Neomycin[7] eye drops. There was increased lacrimation and photophobia since the lesion was not healed.

Case VII: The affected eye showed deep corneal ulceration with Descemetocoele and severe corneal oedema surrounding this defect. Necrotic tissue was noticed around the edges (Plate 7).

Case VIII and IX: Both cases presented moderately deep ulcer on axial cornea surrounded by necrotic tissue.

All these cases required scarification of the surface of the lesion and irrigation with sterile saline to remove the dead and necrotic tissue before applying the collagen diskette.

3.4.4.3. Corneal perforation and staphyloma formation

Case V: The animal was presented with a staphyloma of right eye which was being treated with topical antibiotics. But the fibrin clot was more stable with more amount of adhesions and so the iris could not be reduced and sutured (Plate 8). In this case the surface of the lesion was scarified and the collagen diskette was applied in a manner previously described.

Case VI: The animal was previously treated for corneal erosion for the last two weeks. On the day of surgery, the lesion presented was a fresh staphyloma (Plate 9) and attempt was made to relieve the adhesions and reduce the iris tissue and the edges were sutured using 8/0 braided silk.

[7] Betnesol-N eye/ ear drops(Betamethasone 0.1% and Neomycin 0.5%), GSK. 5 ml bottle.

3.4.4.4. Application of collagen diskette

The diskette was moistened with ciprofloxacin eye drops for about two minutes and kept over the corneal surface (Plate 10). In Case I, on first day, it was left under the covering of the third eyelid and was suspected to have dislodged due to the movement of the third eyelid. Hence to ensure the retention of the material in the eye, the eyelids were closed with temporary tarsorrhaphy using 1/0 braided silk sutures. The collagen diskette was reapplied, on subsequent examination, if the diskette was found completely resorbed, until completion of corneal healing.

3.4.4.5. Tarsorrhaphy

The collagen diskettes available were of the same size and shape and while used in different animals, it was not uniformly fixed over the corneal surface. Hence tarsorrhaphy was performed to keep the collagen diskette in position and the sutures were removed after 72 hours for review of the condition (Plate 11).

3.4.5. Post- operative care

Post operatively, Elizabethan collar was applied to all the animals to prevent self- mutilation and oral administration of anti- inflammatory drug (promethazine theoclate[8]) was also done in cases with severe itching. Antibiotic (ciprofloxacin) and anti-inflammatory (flurbiprofen[9]/ diclofenac[10]) eye drops were instilled four times daily in all cases. In selected cases oral antibiotics (amoxicillin- cloxacillin[11]/ cephalexin[12]) and other supplementary medications

[8] Phenergan, (10mg, 25mg tablets), Nicholas Piramal, Dhar, Madhyapradesh
[9] Flur ,(0.03% w/v), Nicholas Piramal, Dhar, Madhyapradesh. 5ml bottle
[10] Voveran , Novartis India Ltd, Mumbai. 5ml bottle
[11] Novaclox. (amoxicillin 250mg+ cloxacillin 250mg tablets), Novartis.

including multivitamins and minerals were used. Initially used antibiotic was altered only if needed according to the culture and sensitivity results.

3.5. EVALUATION OF THE STUDY

The efficacy of collagen for healing of corneal lesions was assessed by clinical and laboratory studies conducted at intervals *viz.*, on 3rd and 7th days and then fortnightly. The animals were regularly subjected to clinical examination until the cornea became fluorescein test negative or it was followed up to a maximum period of three months. Complications, if, any, observed during the period were also recorded.

3.5.1. Patient evaluation

3.5. 1.1. Physiological parameters

The respiration rate (per minute), pulse rate (per minute) and rectal temperature (°C) were recorded on the day of surgery and on postoperative observation days.

3.5.1.2. General clinical evaluation of the eye

The eye was observed for gross lesions, discharge, vascularity, clarity and the nature of lesion and the site of collagen diskette application.

3.5.1.2.1. Nature of discharge

The presence or absence of ocular discharge and its nature were noticed in all the cases. It was assumed that a purulent discharge indicated presence of infection and additional therapies were indicated.

---

[12] Sporidex DS,(250 mg tablets), Ranbaxy laboratories Ltd., A.P.

3.5.1.2.2. Appearance of corneal lesion after applying collagen

The condition of the lesion, extend and area involved and inflammatory changes were noticed and recorded. Efficacy of collagen for corneal healing was assessed based on these factors.

3.5.1.2.3. Vascularity and redness of cornea

All the operated eyes were examined for the presence of neovascularization until the healing was complete. Advised to continue nonsteroidal anti- inflammatory preparation (flurbiprofen) in these cases.

3.5.1.2.4. Fluorescein dye test

The extend of corneal lesion was assessed by the use of fluorescein dye in the form of sterile impregnated strips. The strips were inserted into the conjunctival sac and kept the lids closed for a few seconds. Afterwards, the strip was removed and excess stain was washed out with saline. Wherever the corneal epithelium was damaged, there was retention of the dye, which gave a bright green fluorescence (Helper, 1989).

3.5.1.2.5. Corneal clarity

The clarity of the cornea including the site of lesion was assessed based on visual examination. The clarity was scored as + (clear), ++ (hazy), +++ (moderate opacity) and ++++ (complete opacity).

3.5.1.3. Exfoliative cytology of lacrimal smear

The lacrimal fluid was absorbed onto moist cotton swabs and rolled over the slide to prepare the smear. It was then air- dried and stained with Wright's

stain to demonstrate the exfoliated cells including epithelial cells, anuclear cells and microbes, if any, according to the method described by Gelatt (1991).

3.5.1.4. Visual function tests

The visual function of the affected animals was assessed based on the menace reflex and pupillary reflex. In severely affected corneas this method was not effective due to extensive corneal oedema and keratitic changes.

3.5.2. Evaluation of the collagen diskette

3.5.2.1. Absorption of the material

The collagen diskette used for the present study was thought to be completely absorbed within 72 hours according to the information provided by the CLRI, who supplied the material. The region of collagen diskette applied was thoroughly examined for the presence of the collagen residue, nature of the lesion/ healing state, clarity of the region and any other changes.

3.5.2.2. Adverse reactions/ patient compatibility

Signs like increased vascularity, corneal oedema and ocular discharge *etc.* were observed if present and, considered it as an undesirable reaction to the material.

3.5.2.3. General appearance of the cornea

The overall appearance of the cornea was regularly noticed and recorded. Variations from the normal like denting, ulceration and healing at the site were recorded.

3.5.3. Complications encountered

3.5.3.1. Infection

In all the cases, examination was conducted postoperatively to identify the presence of infection. Purulent discharge, redness and discomfort were considered to be indicating infection.

3.5.3.2. Mutilation

In all the animals under study, Elizabethan collar was applied to prevent self- mutilation. The chance of mutilation and subsequent complications were considered and any discomfort and irritation to the operated area were minimized with the use of anti histaminics or other nonsteroidal anti-inflammatory drugs.

3.5.3.3. Others

Other complications associated with the conditions under study were pigmentation, dryness of cornea, and corneal opacity which were also recorded. The pigmentation was treated with gentamicin-hydrocortisone[13] or betamethasone-neomycin eye drops.

[13] Genticyn HC, (Gentamicin sulphate 0.3%, Hydrocortisone acetate 1%), Allergan.

Plate 1. **Sterile collagen diskette**

Plate 2. **Fluorescein impregnated strips and antibiotic used for soaking the collagen diskette**

Plate 3. **Preoperative appearance of the cornea with lacerated wound - Case No. I**

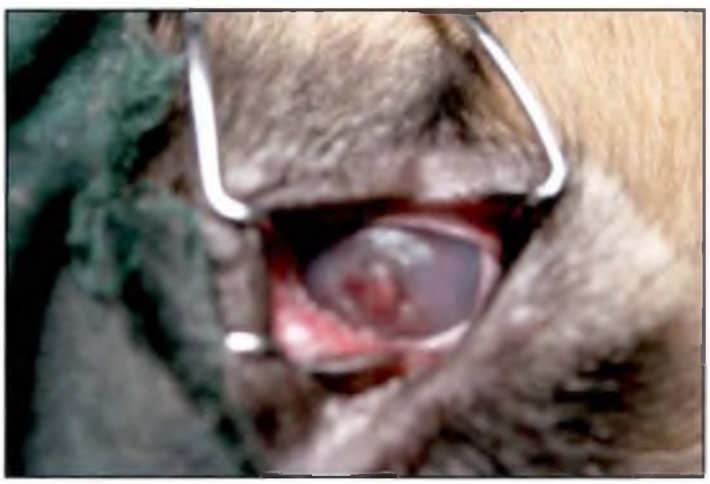


Plate 4. **Lesion in plate 3 after removal of fibrin clot and debridement - Case No. I**

Plate 6. **Preoperative appearance of the cornea with corneal ulcer - Case No. III**

Plate 7. **Preoperative appearance of the cornea with ulceration and descemetocoele formation - Case No. VII**

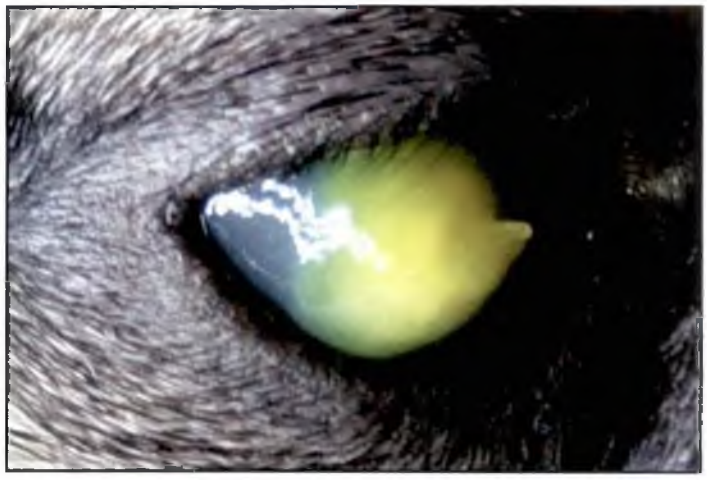


Plate 8. **Preoperative appearance of the cornea with staphyloma**

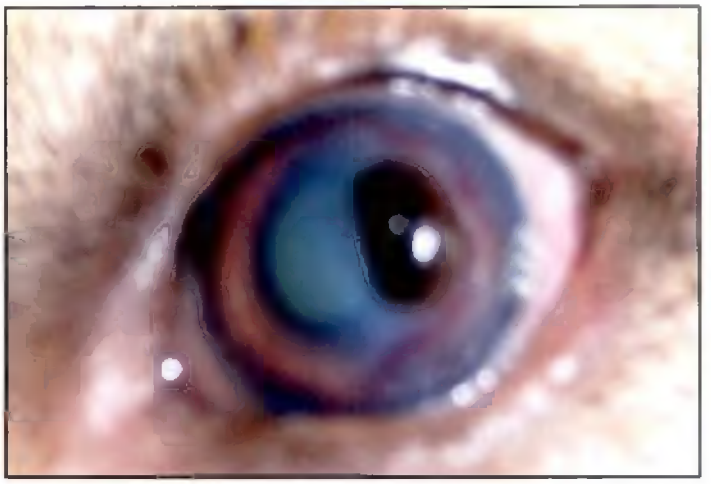


Plate 9. **Preoperative appearance of the cornea with fresh staphyloma formation - Case No. VI**

Plate 10. **Moist collagen diskette over the corneal lesion - Case No. I**

Results

4. RESULTS

4.1. SELECTION OF CASES

All the animals presented to the hospital with affections of eye were subjected to detailed clinical examination and nine cases (8 dogs) with corneal lesions were selected for the study. In all the animals the collagen graft was applied over the lesion after surgical management according to the conditions presented.

4.2. ANAMNESIS

Among the eight dogs selected four were males and the remaining were females. The age of dogs ranged from 3.5 months to 24 months with an average of 14.44 months. The dogs belonged to different breeds *viz.*, Pug (5), German Shepherd Dog (1), Spitz (1) and Labrador Retriever (1). The corneal lesions were more predominantly noticed in pugs (55.5 %) (Table 1). All the pugs were of seven months age except one puppy. The exciting cause for lesion was not obvious in any of the cases due to inadequate history. But involvement of traumatic injury was suspected in almost all cases. Of the nine eyes studied, the right eye was affected in four, and the left eye in five cases.

4.3. TYPES OF LESIONS

Lesions under study were on the axial or peripheral cornea. They included corneal injury (Two), ulceration (Four), ulceration with Descemetocoele (One) and perforation with staphyloma formation (Two). In addition to these, other lesions like periorbital dermatitis and swelling and proptosis were found in two dogs which were suspected to be the major cause for corneal injury on account of self- mutilation and exposure of cornea.

4.4. EVALUATION OF THE TECHNIQUE

4.4.1. Anaesthetic protocol

All the animals were subjected to general anaesthesia for surgical treatment. Anaesthetic protocol including, atropine sulphate (0.045mg/ kg body weight) and xylazine hydrochloride 2mg/ kg body weight for premedication, ketamine hydrochloride 5mg/ kg body weight for induction and equal volumes of xylazine and ketamine intravenously to effect for maintenance of anaesthesia was found sufficient for manipulations on the cornea. On review of cases for subsequent observations, sedation with xylazine was used only in animals, which were difficult to handle. In all the cases, topical anaesthetic of choice was Lignocaine hydrochloride (4%) for simple debridement and cleaning of the eye.

4.4.2. Suturing of the corneal wounds

Suturing of the corneal laceration/ perforation was needed in cases I, and VI. It was performed according to the microsurgical principles using braided silk of size: 8/0. In Case No. I, edges could not be apposed completely due to the lacerated nature of the wound and the sutures were provided to offer some support and to prevent further gaping of edges. In Case No. VII, corneal rupture of the ulcerated area with staphyloma was noticed, and this was reduced after thorough instillation with antibiotic and sutured the edges. The sutures, in Case No. I, were removed after 2 weeks period. In the other two cases, the sutures were found to be disrupted on review and partial healing was obtained.

4.4.3. Application of collagen diskette

Difficulty was encountered in application and retention of the collagen diskettes. It should be applied in such a manner that it should be in flush with the

corneal surface without any air space inside. It is difficult to use flat diskettes over canine corneal surface with varying degrees of curvature. The edges have to be cut in 'v' shape, and adjusted to avoid the space inside. Tarsorrhaphy was needed to ensure the retention of the material due to the chance of dislodgement of diskette by the movement of third eyelid.

4.4.4. Post- operative care

All the animals were provided with Elizabethan collars until proper healing of the cornea in order to prevent self-mutilation and were found to be very effective. The use of amoxicillin- cloxacillin/ cephalexin orally was advised for the first five to seven postoperative days and ciprofloxacin topically, to be continued till complete healing of the lesion and was found to be effectively counteracting infections. flurbiprofen/ diclofenac were prescribed for topical use until the inflammation subcided and vascularity/ pigmentation was resolved.

4.4.5. Removal of tarsorrhaphy sutures

The tarsorrhaphy was done primarily for the retention of collagen diskette. It also provided support to the cornea during healing period and left sufficient space for administration of topical medications. The sutures were removed on review of the cases and reapplied only when the collagen diskette was replaced. In Case No. VII, severe sepsis was noticed on review on third day and so collagen was not reapplied but tarsorrhaphy was continued.

4.5. PATIENT EVALUATION

4.5.1. General clinical condition (Table 1)

All the animals except Case No. IV were found to be in very good or excellent condition at the time of presentation of the cases. The puppy, in Case No.

IV was found to be very weak and anaemic on examination. Some injury had occurred about two weeks back and the animal was presented in a bad condition. Vitamin and mineral supplementations were provided for all those animals (Case nos. III, IV, V and VI), which were suspected for having these deficiencies.

4.5.2. Physiological parameters (Table 3)

4.5.2.1. Respiration rate

The average respiration rate of animals was found to be 32.15± 0.16 preoperatively on the day of surgery. It was 33.25±1.06, 29.68± 0.84, 30.18± 2.26, 29.80± 1.08 and 28.64± 0.24 on 3rd, 7th, 14th, 28th and 60th postoperative days respectively. All values were within the normal range.

4.5.2.2. Pulse rate

The mean pulse rate recorded was 118.80± 2.69 preoperatively on the day of surgery, and 98.54± 2.62, 86.00± 5.42, 89.60± 2.64, 104.04± 0.46, and 96.24± 2.24 and 3rd, 7th, 14th, 28th and 60th postoperative days respectively and the values were found to be in the normal range.

4.5.2.3. Rectal Temperature

The mean rectal temperature recorded was 39.15± 0.16 preoperatively on the day of surgery, and 38.75± 1.05, 39.13± 0.14, 38.80± 0.24, 39.08± 0.20 on 3rd, 7th, 14th, 28th and 60th postoperative days respectively. All the values were within the normal range.

4.5.3. Visual function evaluation

In cases I, VI and IX, animals were found to have normal menace and pupillary reflexes. In others, visual reflexes could not be assessed due to severity of corneal oedema and opacity. By 28th postoperative day the opacity was much reduced and normal visual reflexes were reestablished in all the cases except in case nos. IV and VII, in which other complications were observed.

4.5.4. Fluorescein dye test (Table 4)

Fluorescein impregnated strips were used for assessing the depth and extend of corneal lesions. On first day all the eyes showed retention of fluorescein dye (Plate 12). On seventh day, except Case Nos. V and VI all others were either fluorescein test negative or with negligible level of retention (Plate 13) and in all the cases dye retention was absent by next review on 14th day.

4.5.5. Vascularization of cornea (Table 5)

Vascularization of cornea developed in six cases, including case nos. I, III, V, VI, VII and VIII during the later phase of healing. Case No. III, V and VI (Plate 14) showed vascularity by third day and it was found increased on seventh day onwards. The degree of vascularization decreased gradually and disappeared by 60th day except in Case No.I. In, Case No. I, maximum degree of vascularization was seen on 14th day postoperatively and reduced by 28th day. The entire cornea became devoid of vascularization by the end of first month itself.

4.5.6. Corneal clarity (Table 6)

All the operated eyes were examined regularly and the corneal clarity was recorded as + (clear), ++ (hazy), +++ (moderate opacity) and ++++ (complete opacity). In three cases I, III and VI the degree of opacity was found to increase on

third day after surgery. In three Case nos. (II, VII and VIII) there was improvement in corneal clarity and in case nos. III, V (Plate 15) and IX there was no variation in the corneal clarity on seventh day and increase in corneal opacity was noticed in Case No. I and IV. In all the other cases there was not much improvement in the clarity at the site of lesion.

By 14^{th} day, except in Case Nos. II and V(Plate 16), all eyes became clear at the uninjured part and these two cases also regained normal clarity by 28^{th} day. The injured area regained clarity by varying periods in each case. In Case No. I, haziness remained after three months (Plate 17). Case Nos. II and III regained clarity at 28^{th} postoperative day. Case Nos. II, III and V (Plate 18) became clear with a small scar at the center by 3 months period. Case VI also had an area of opacity with iridal adhesions at the center of injury by 30 days (Plate 19). There was pigmentation and cloudiness in cases VII and VIII at the limbal margin.

4.6. HAEMATOLOGICAL EVALUATION

Whole blood samples were examined preoperatively in all the cases(Table 7).

4.6.1. Haemoglobin concentration

In the cases studied, average haemoglobin concentration (g/dl) was found to be 12.47 ± 0.54 g/dl. All the values, except in Case No. IV, were in normal range. In this animal, the value was slightly lower.

4.6.2. Volume of packed red cells

The average volume of packed red cells (%)recorded was, 31.63 ± 0.85% which was also found to be normal. Case No. IV gave a lower value as compared to others.

4.6.3. Total leucocyte count

The total leucocyte count (10^3/ cu.mm) was also estimated in all the samples collected. The average of value obtained on analysis was, 10.57 ± 1.26 which showed not much significant variation among animals under study.

4.6.4. Differential leucocyte count

In all the animals, the average neutrophil count (%) was found to be 78.13 ± 0.64 %. In cases IV, V and VII the value was slightly higher than normal. The average eosinophil count was 2.50 ± 0.06 % and the average lymphocyte count was 18.75 ± 1.24 % in these animals. Average monocyte count was 3.20 ± 0.04 %. Basophils were absent in all the samples examined.

4.7. CULTURE AND SENSITIVITY OF CORNEAL SWAB (Table 8.)

In this study Ciprofloxacin was primarily selected as the antibiotic for topical therapy. The drug was altered only on the basis of culture and sensitivity result whenever needed. In cases II, VI and IX no growth was obtained after incubation for 24 hours. Gram positive cocci were isolated in all the other cases and Gram negative bacilli were concurrently isolated in Case No. IV. Based on the sensitivity results, ciprofloxacin was selected for therapy in cases I, II, III, and VI, chloramphenicol in IV and V and gentamicin in VII, VIII and IX.

4.8. EXFOLIATIVE CYTOLOGY (Table 9)

The exfoliated cells were collected, smears were prepared, stained with Wright's stain and examined. The different cell types observed in the smears are:

4.8.1. Nucleated epithelial cells

In all the cases, nucleated epithelial cells were seen in a higher quantity, at the initial phase of the healing of corneal lesion (Plate 20). Their number diminished gradually and was negligible at the end stages of healing in all cases.

4.8.2. Anuclear keratinized cells

In all the cases anuclear cells were seen before healing was completed. The number of these cells was found to be increasing by third or seventh day in all cases. In, Case No. VII, it was recorded to be the maximum among all cases on the third day.

4.8.3. Polymorphonuclear cells

Polymorphonuclear cells were observed in Case No. I, on the first day in very high numbers, and decreased to negligible levels, by third day after surgery (Plate 21). This type of cells was not observed in any of the other cases, either before or after surgery.

4.9. EVALUATION OF THE COLLAGEN DISKETTE

4.9.1. Absorption of collagen (Table 10)

In all the cases the collagen diskette was found to have completely dissolved by third day. There was no remnant appreciable on the corneal surface. Only in Case No. I, when the collagen was applied for the first time, the retention could not be ensured and hence assessment of absorption was also not reliable. Hence it was reapplied on the third day and was completely absorbed by the 7^{th} postoperative day.

4.9.2. Patient comfort

All the animals tolerated the collagen diskette applied. No animal was reported to have any adverse reaction to the material. Excess discharge, oedema or redness indicating an adverse reaction was absent in all the cases studied.

4.9.3. Complications

4.9.3.1. Infection

Only in Case No. VII, presence of infection was observed. There was sepsis of the eyeball (on third day) and on removal of the pus inside, some part of the vitreous was also removed. Use of proper antibiotic (cephalexin orally and gentamicin topically) based upon the culture and sensitivity results completely resolved the infection within four days.

4.9.3.2. Mutilation

Mutilation of the sutures was noticed in cases IV, VI and VII. In cases IV and VII, the tarsorrhaphy sutures were found to have ruptured on third day. In Case No. VI, animal was reported to exhibit severe scratching of the eye for about five days after initiation of treatment, but the sutures were intact. The eyelids were found inflamed.

4.9.3.3. Others

4.9.3.3.1. Pigmentation

In Case No. I, pigmentation of the cornea developed from the limbus close to the injured area. After removing the sutures and complete healing of the cornea, it was advised to continue the use of topical anti-inflammatory agent (flurbiprofen).

On 60th day, it showed much improvement and the pigmentation was completely resolved in three months period (Plate 22). In Case No. IV, proptosed eye became dry and exposure keratitis developed followed by pigmentation and keratinization of the superficial cornea. Cornea became totally opaque and further evaluations became impossible. Pigmentation was seen in Case Nos. VII and VIII (same animal) (Plate 23) and the animal was treated with gentamicin- hydrocortisone combination. The progress of pigmentation was found to have arrested within seven days.

4.9.3.2. Dry cornea/ Opacity/ Ruptured corneal sutures

In Case No. IV dry cornea developed probably due to prolonged exposure ever since the injury occurred. Rupture of corneal sutures was observed in cases VI and VII.

Table 1. Anamnesis of cases with corneal lesions selected for the clinical study

Case. No:	Breed	Age (months)	Sex	Condition of the eye	Duration of illness	Previous medications
I	German Shepherd Dog	8	M	Deep corneal injury	1 day	Nil
II	Pug	7	F	Corneal ulcer	2 days	Nil
III	Spitz	18	F	Corneal ulcer	1 week	Had been treated with Betnesol eyedrops
IV	Pug	3.5	F	Proptosis/ Corneal injury	2 weeks	Reduced proptosis 3 days prior to surgery and was treated with Chloramphenicol – Dexamethasone combination
V	Pug	7	F	Staphyloma	2 days	Treated with penicillin, Streptomycin, Sulfamerazine and Hydrocotisone combination and Chloramphenicol ointments
VI	Labrador	24	M	Staphyloma	1 day.	Erosion seen for 2 weeks Treated with chloramphenicol
VII	Pug	7	M	Corneal ulcer and Descemetocoele	5 days	Treated with chloramphenicol, polymyxin B sulphate and dexamethasone combination
VIII	Pug	7	M	Corneal ulcer	5 days	Treated with chloramphenicol, polymyxin B sulphate and dexamethasone combination
IX	Pug	7	F	Corneal ulcer	4 days	Treated with chloamphenicol

Table 2. General clinical observations on the eye of the cases under study

Case No:	Eye Affected Right (R)/ Left (L)	Eyelid	Conjunctiva	Cornea	Sclera	Eyeball
I	R	Normal	Congested	Fresh, deep corneal injury; Corneal opacity	Normal	Normal
II	R	Normal	Normal	Corneal ulcer; Macula	Normal	Bulged out
III	L	Inflamed, Erythematous	Congested	Corneal ulcer over a wide area; Corneal opacity	Normal	Normal
IV	L	Normal	Swollen	Proptosis/ Corneal injury Dry cornea	Episcleral congestion	Proptosed
V	R	Normal	Congested	Staphyloma; Corneal opacity	Normal	Bulged out
VI	L	Inflamed, Swollen	Congested	Fresh staphyloma; Periorbital dermatitis	Normal	Normal
VII	R	Normal	Normal	Descemetocoele; Corneal opacity	Normal	Enlarged
VIII	L	Normal	Normal	Deep corneal ulcer; Corneal opacity	Normal	Normal
IX	L	Normal	Normal	Moderately deep corneal ulcer; Corneal opacity	Normal	Normal

R Right eye
L Left eye

Table 3. Physiological parameters (Mean ± SE) before and after surgery

Parameters	Before surgery	Postoperative days				
	0day	3	7	14	28	60
Respiration rate (per minute)	32.15± 0.16	33.25± 1.06	29.68± 0.84	30.18± 2.26	29.80± 1.08	28.64± 0.24
Pulse rate (per minute)	118.80± 2.69	98.54± 2.62	86.00± 5.42	89.60± 2.64	104. 04± 0.46	96.24± 2.24
Temperature (°C)	39.15± 0.16	38.75± 1.05	39.13± 0.14	38.80± 0.24	39.08± 0.20	38.25± 0.18

Table 4. Observations on fluorescein dye retention before and after surgery

Case No:	Before surgery	Postoperative days of observation					
	0day	3	7	14	28	60	90
I	+	+	-	-	-	-	-
II	+	+	+/-	-	-	-	-
III	+	+	+/-	-	-	-	-
IV	+	-	-	-	-	-	-
V	+	+	+	-	-	-	-
VI	+	+	+	-	-	-	-
VII	+	+	+/-	-	-	-	-
VIII	+	+	-	-	-	-	-
IX	+	+/-	-	-	-	-	-

\+ Present
\- Absent
+/- Faint

Table 5. Observations on the vascularization of cornea before and after surgery

Case No:	Before surgery	Postoperative days of observation					
	0 day	3	7	14	28	60	90
I	-	-	-	++	+	-	-
II	-	-	-	-	-	-	-
III	-	+	++	+	+/-	-	-
IV	-	-	-	-	-	-	-
V	-	+	++	+	+/-	+/-	-
VI	-	+	++	+	+	-	-
VII	-	-	+	-	-	-	-
VIII	-	-	+	-	-	-	-
IX	-	-	-	-	-	-	-

+ Present
- Absent
+/- Faint capillaries

Table 6. Observations on the degree of corneal clarity at the site of lesion before and after surgery

Case No:	Before surgery	Postoperative days of observation					
	0 day	3	7	14	28	60	Remarks on 90th day
I	++	+++	+++	++	++	++	Hazy
II	++++	+++	+++	++	++	+	Clear
III	++	+++	++	+	+	+	Clear
IV	+++	+++	++++	++++	++++	++++	Opaque
V	+++	+++	+++	++	+	+	Clear with small scar
VI	++	+++	+++	++	++	++	Scarred at the center
VII	+++	++	++	++	++	+	-
VIII	+++	++	++	++	++	++	-
IX	++	++	++	+	+	+	-

Corneal clarity denoted as:
+ clear
++ hazy
+++ moderate opacity
++++ complete opacity

Table 7. Preoperative observations on the haematological parameters of the cases studied

Parameters		Animals under study							
		I	II	III	IV	V	VI	VII&VIII	IX
Haemoglobin (g %)		13.4	12.8	11.8	9.6	12.0	14.4	12.8	13.2
Volume of Packed Red Cells (%)		38	31	30	28	32	39	32	33
Total leucocyte count (10^3/ cu.mm)		9.80	10.28	10.18	11.16	10.90	10.82	11.26	10.15
Differential leucocyte count (%)	Neutrophil	72	78	73	84	86	73	81	78
	Eosinophil	3	-	4	2	2	3	3	2
	Lymphocyte	24	21	22	14	12	22	16	19
	Monocyte	1	1	1	-	-	2	-	1
	Basophil	-	-	-	-	-	-	-	-

Table 8. Observations on the culture and sensitivity of corneal swabs from the cases studied

Case No:	Culture and sensitivity results			Antibiotic selected
	Organism cultured	Sensitive to	Resistant to	
I	Gram positive cocci	Chloramphenicol 2+ Ciprofloxacin 3+ Gentamicin 2+ Amikacin 2+	Penicillin Oxytetracycline Amoxycillin	Ciprofloxacin
II	No growth	-	-	Ciprofloxacin
III	Gram positive cocci	Cefotaxim 3+ Amoxycillin 3+ Penicillin 2+ Cotrimoxazole 2+ Ciprofloxacin 3+	Pefloxacin Oxytetracycline	Ciprofloxacin
IV	Gram negative bacilli and Gram positive cocci	Cefotaxim 3+ Ciprofloxacin 2+ Ampicillin 3+ Gentamicin 2+ Chloramphenicol 3+	Penicillin Cotrimoxazole Oxytetracycline	Chloramphenicol
V	Gram positive cocci	Cefotaxim 3+ Chloramphenicol 3+ Gentamicin 2+ Ampicillin 2+	Pefloxacin Oxytetracycline	Chloramphenicol
VI	No growth	-	-	Ciprofloxacin
VII	Gram positive cocci	Ceftriaxone 3+ Gentamicin 3+ Amoxycillin 2+	Penicillin Oxytetracycline	Gentamicin
VIII	Gram positive cocci	Ceftriaxone 3+ Gentamicin 3+ Amoxycillin 2+	Penicillin Oxytetracycline	Gentamicin
IX	No growth	-	-	Gentamicin

Table 9. Observations on exfoliative cytology of lacrimal smear before and after surgery

Case No:		I			II			III			IV			V			VI			VII			VIII			IX		
Exfoliate cell types		E	A	N	E	A	N	E	A	N	E	A	N	E	A	N	E	A	N	E	A	N	E	A	N	E	A	N
Before surgery	0 day	++	+	++++	+	+	-	++	+	-	+	-	-	+++	++	-	++	++	-	+	++	-	-	+	-	+	++	-
Postoperative day of observation	3	+	++	-	+	++	-	+	+	-	+	++	-	+	+	-	+	++	-	++	++++	-	+	+	-	+	+	-
	7	+	++	-	+	++	-	+	-	-	+	++	-	-	++	-	++	+	-	++	++	-	+	-	-	-	-	-
	14	-	+	-	+	+	-	+	-	-	+	+	-	+	++	-	+	++	-	+	+	-	-	-	-	+	-	-
	28	+	-	-	+	-	-	+	-	-	-	++	-	-	+	-	+	+	-	-	-	-	+	-	-	+	-	-
	60	+	-	-	-	-	-	-	-	-	+	++	-	+	-	-	_	+	-	+	-	-	+	-	-	-	-	-

E Epithelial cells

A Anuclear keratinized cells

N Neutrophils

Table 10. Observations on the collagen absorption during the postoperative period.

Case No:	Days of observation			
	Stage of absorption		Stage of healing	
	3rd	7th	3rd	7th
I	Complete & reapplied	Complete	Healing	Epithelialization complete; Hazy
II	Complete	-	Healing	Healing; Area of opacity reduced
III	Complete	-	Healing	Healing; Degree of opacity reduced
IV	Complete	-	Epithelialization complete	Healed; Cornea pigmented
V	Complete & Reapplied	Complete	Healing	Epithelialization complete; Slight denting
VI	Complete & reapplied	Complete	Healing	Healing; Vascularized
VII	Complete	-	Not healing; Sepsis noticed	Healing
VIII	Complete	-	Healing	Partially healed; Area of opacity reduced
IX	Complete	-	Healing	Healing

Table 11. Observations on the appearance of cornea during the postoperative period.

Case No:	Days of observation					
	3rd	7th	14th	28th	60th	90th
I	Healing	Epithelialization Complete; Hazy	Healing; Hazy; Sutures intact	Healing; Pigmentation seen	Completely healed & hazy	Hazy at the injured area
II	Healing; moderate opacity	Area of opacity reduced	Epithelialization complete; Haziness	Small area of haziness	Completely healed& Clear	Clear
III	Depth of ulcer reduced; moderate opacity	Partially epithelized; Low degree of opacity	Epithelialization complete; Healing	Healing	Healing complete; Clear	Clear
IV	Injury healed; Pigmentation noticed	Pigmentation& keratinization; High intra ocular pressure	Dry & pigmented	Dry & pigmented	Dry & pigmented	Dry & pigmented
V	Opacity persists; Partially epithelized	Epithelialization complete; Opaque center with slight denting	Exuberant granulation along edges	Granulation regressed ; improved clarity	Completely healed with small scar	Very small scar at the center

Table 11.Continued

Case No:	Days of observation					
	3rd	7th	14th	28th	60th	90th
VI	Vascularization noticed; Injured area reddish	Healing; Vascularization at the site of lesion	Moderate opacity; Iridal adhesion; Epithelialization complete	Area & extend of opacity reduced	Opacity much reduced; Vascularity resolved	Healing complete with scar at the center
VII	Staphyloma noticed; Fibrin clot protrudes out	Sutures ruptured; Injured area opaque; Epithelialization partial	Epithelialization complete; small area of opacity at the center; Pigmentation	Axial cornea cloudy; Pigmentation along the periphery	Pigmentation arrested	-
VIII	Healing in progress; Area & extend of opacity reduced	Epithelialization complete; Opacity persists	Small area of low degree of opacity; Pigmentation	Axial cornea clear; Pigmentation along the periphery	Pigmentation arrested	-
IX	Healing; Corneal injury ventro- medially	Epithelialization complete ; small scar; Hazy	Small area ofhaziness at the center	Clear	Clear	-

Table 12. Observations on the complications encountered during the postoperative period

Complications	Cases studied								
	I	II	III	IV	V	VI	VII	VIII	IX
Infection	-	-	-	-	-	-	Sepsis of eyeball. Part of the vitreous expressed out	-	-
Mutilation	-	-	-	Tarsorrhaphy sutures disrupted	-	Scratching of the eye	Tarsorrhaphy sutures disrupted	-	-
Others	Graft retention not confirmed	-	Erythematous lid lesions. Scratching resolved	Exposure keratitis, pigmentation & keratinization	Excessive granulation on 15th day, which resolved later	Corneal sutures disrupted	-	-	-

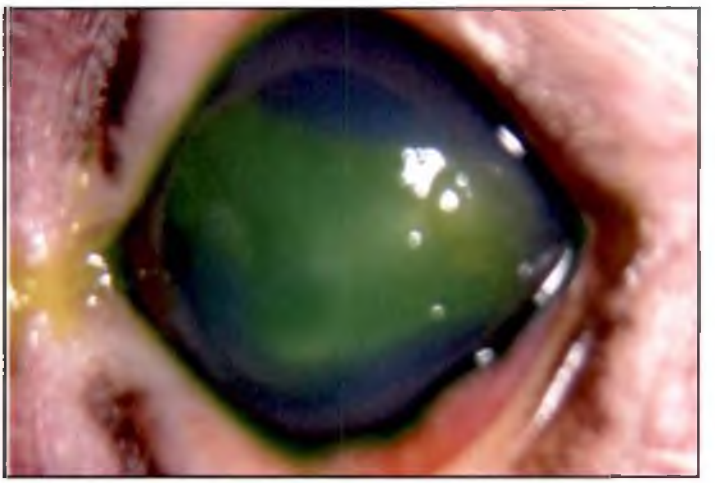
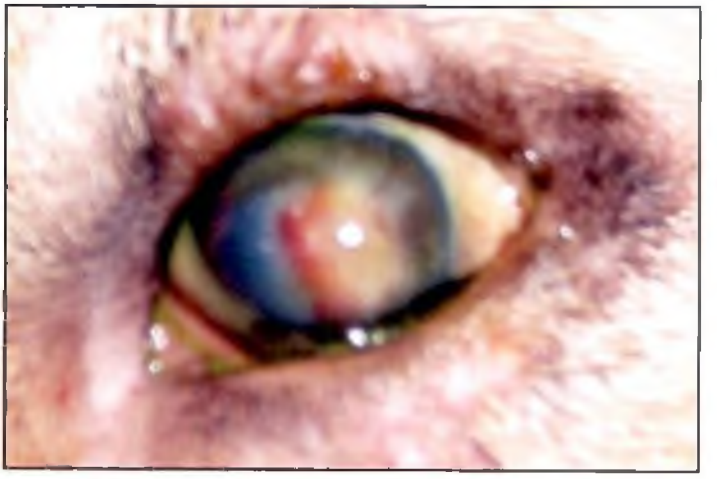


Plate 12. **Preoperative appearance of the cornea with corneal ulcer showing fluorescein dye retention - Case No. III**

Plate 13. **Completed epithielialization and absence of fluorescein dye retention on 7th day - Case No. I**



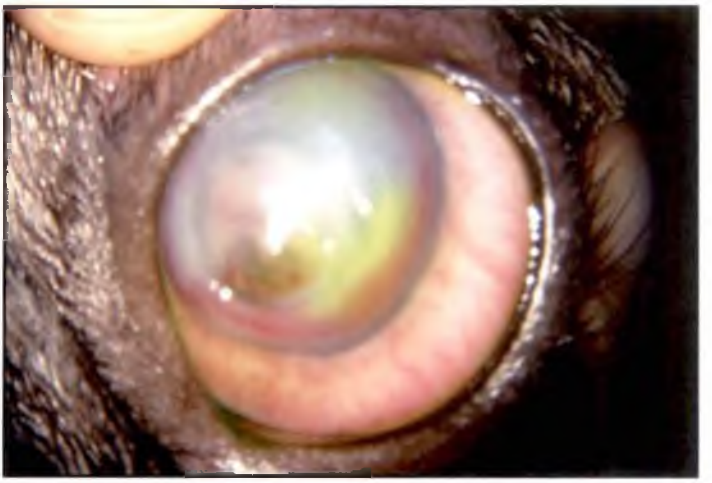


Plate 15. **Corneal opacity and vascularization on 7th day - Case No. V**

Plate 16. **Increased corneal opacity on 7th day - Case No. IV**

Plate 18. **Clarity of the cornea with small scar after 3 months- Case No. V**

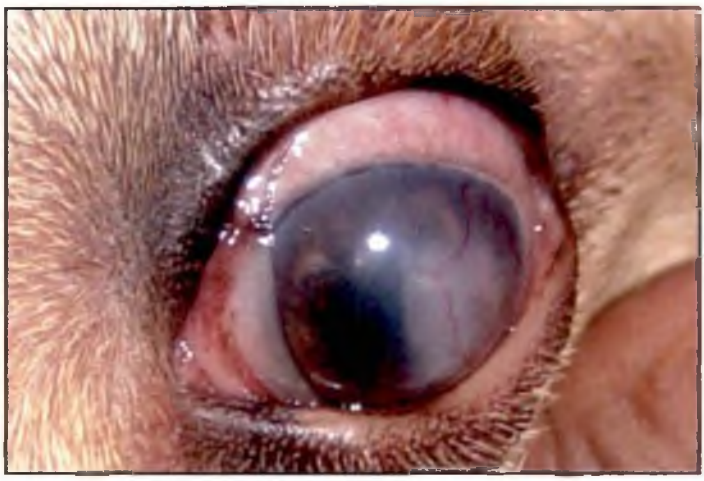
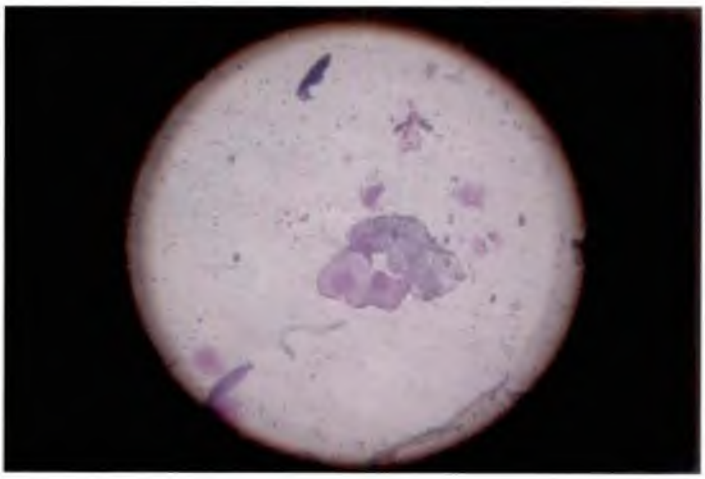


Plate 19. **Central scar and haziness of the cornea with some vascularity - Case No.VI**



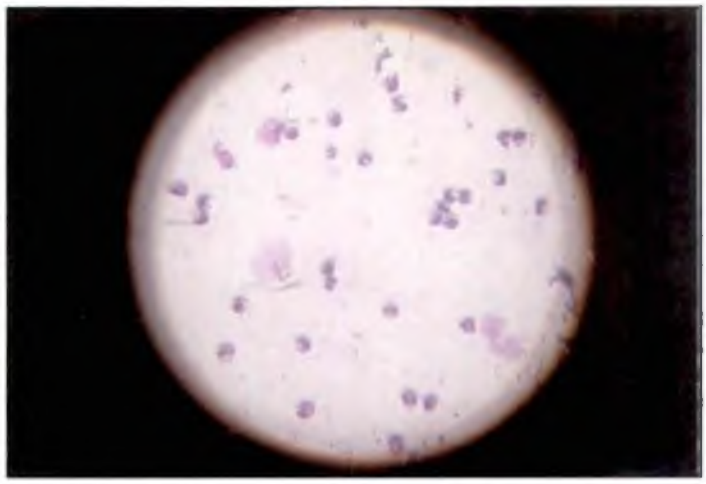


Plate 21. **Exfoliative cytology showing large number of polymorphonuclear cells**

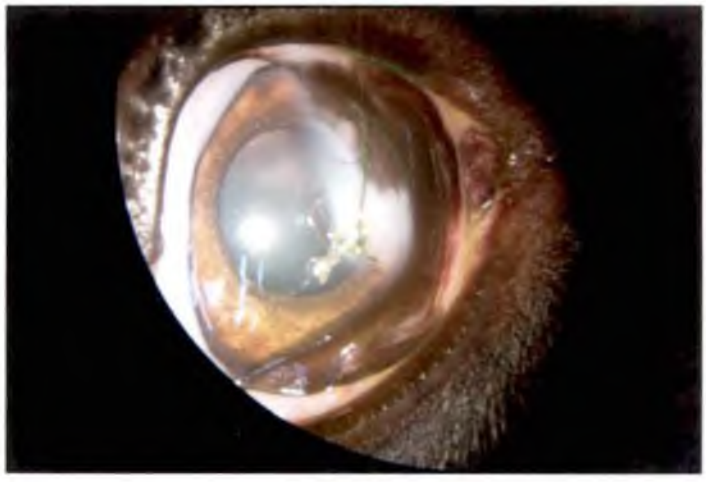
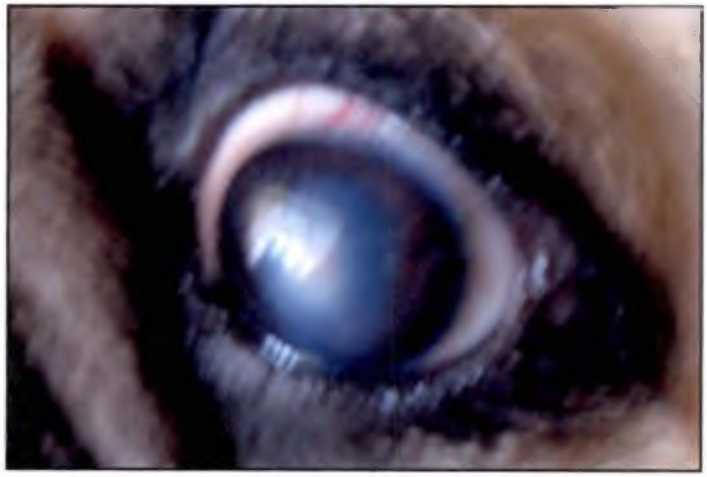


Plate 22. **Pigmentation of the cornea on 14th day - Case No. I**



Discussion

5. DISCUSSION

Corneal lesions are of a frequent occurrence in dogs, which, may lead to many complications affecting vision and hence, the quality of life of the animal. The traditional treatment modalities of such affections are prolonged both in medical and surgical cases. The use of collagen is reported to be having a favourable effect on the healing of tissues. Hence this study was conducted to evaluate the effectiveness of a collagen diskette prepared specially for ophthalmic use in the treatment of such lesions in dogs. Nine hggcases were analyzed for the etiology, predisposing factors, healing process, effect of collagen, therapeutics and complications etc.

5.1. SELECTION OF CASES

The dogs presented to the Veterinary hospitals, Mannuthy and Kokkalai, College of Veterinary and Animal Sciences, with the history of corneal lesions were selected for the study. These included fresh injuries and older lesions not responding to other medical treatments.

5.2. ANAMNESIS

Among eight animals in which the study was conducted (one dog was having bilateral affection of the cornea), two dogs were adults and all others except one were juveniles and the remaining was a puppy. There were no specific reports regarding occurrence of corneal lesions in dogs of different age. But it can be assumed from the results of the study that juveniles due to their active and restless nature might be encountered with such lesions produced mostly by traumatic insults. Breeds affected included, Pug (5), German Shepherd Dog (1); Spitz (1) and Labrador Retriever (1). This result is in accordance with the observations of Startup (1984) and Whitley *et al.* (1995) who reported a higher rate of incidence of corneal lesions in brachycephalic breeds of dogs including pug. Whitley *et al.* (1995) has

described various eye affections in dogs seen specifically in pugs. Of the 9 eyes studied, both the right (4) and left (5) were involved.

5. 3. TYPES OF LESIONS

The lesions included corneal injury (2, one associated with proptosis), ulceration of cornea (4) and corneal perforation with staphyloma formation (3). The inciting cause of these lesions was not very clear in any of the cases. However, a traumatic insult was thought to be the probable cause in most of them as described by Whitley (1991). Other factors like influence of breed and age support this inference. Wilcock (1993) and Michau *et al.* (2003) have conducted studies on corneal ulcers and their healing which explains same factors affecting the process. Shivley and Epling (1970) reported a relation between canine corneal lesions and the germ layer of origin which needs more extensive histological analysis.

5.3.1. Corneal ulcer

Corneal ulcer was reported in five cases in which one was associated with a descemetocoele. The clinical findings in this study revealed that the brachycephalic breeds, like pug are more prone to corneal injury and subsequent infection which is in accordance with the observations of Startup (1984) who explained that the avascular nature of cornea and lowered sensitivity may be contributing to the occurrence of corneal ulcers in breeds like pug. However, his explanations that these breeds are more susceptible due to an inherited corneal insufficiency and a lack of protective eye consciousness and, the possibility of nerve deficiencies affecting the central corneal areas has to be analyzed thoroughly.

5.3.2. Proptosis

Proptosis observed in one case was presented for treatment only after two weeks, which affected the nature of cornea and other structures. This was substantiated by the opinion of Gilger *et al.* (1995) that early treatment is the method to resolve swelling and inflammation earlier and potentially decrease permanent damage to the eye. Broad spectrum antibiotics were advised to reduce the risk of infection and tarsorrhaphy was recommended after reduction where the degree of proptosis is pronounced which was found useful to a limited extent in this study. The observation of Bedford (1987); Kern (1991) and Gilger *et al.* (1995) revealed it as a quite common condition among the brachycephalic breeds, and the amount of force also need not be considerable. After successful repair, some degree of proptosis may persist which was seen in Case No. IV.

5.3.3. Corneal injury

Two cases of corneal injury were studied, of which one was a fresh, deep injury and the other was a superficial one associated with proptosis. The deep injury was sutured in partial thickness pattern which was found to be effective in preventing gaping and promote healing. Deep corneal ulceration is usually accompanied by marked corneal oedema and there may be stromal vascularization (Bedford, 1987) as evidenced by the clinical findings. In the second case, superficial injury was caused by prolonged exposure followed by proptosis. The condition was complicated by the delay in treatment at the initial stage itself.

5.3.4. Perforation and staphyloma formation

Staphyloma formation was noticed in two cases. In only one case (Case No. VI) adhesions were relieved effectively and the corneal edges could be sutured which supports the report of Startup (1984) who explained the

difficulties encountered in treatment of staphyloma. The perforation injuries were difficult to treat because of the increased chance of adhesion and infection. Sansom (2000) suggested that the aqueous clot should not be removed prior to general anaesthesia and surgical repair to avoid early disruption of the clot and collapse of anterior chamber.

5.4. SELECTION OF TREATMENT PLAN

5.4.1. Direct suturing of corneal wounds

Deep and uncomplicated corneal lacerations could be sutured directly as per Glover (2000). In corneal perforations the prolapsed iris has to be reduced or resected out and the cornea closed.

5.4.2. Debridement/ Scarification of corneal wounds

For treatment of ulceration, cleanliness of the cornea and conjunctival sac is essential and is best accomplished by the use of saline irrigations and was found effective. Necrotic tissue present often requires debridement under general anaesthesia and in some cases, de- epithelialization was also indicated. In this study, this principle was followed in seven cases and was successful. Broad spectrum antibiotic (ciprofloxacin) was recommended for initial use and over-treatment with antibiotics was avoided which may interfere with corneal regeneration. Topical application of corticosteroid is suggested to be permissible in the control of unwanted vascularization once the healing process is complete and the cornea is fluorescein negative. Saline irrigations are reported to be useful for the treatment of corneal ulcers in order to remove the dead and necrosed tissue. Paracentesis of the anterior chamber was performed in one case, as it was suggested in case of threatened perforation or deep ulceration or where there is increased intraocular tension.

5.4.3. Collagen diskettes

The collagen diskettes were prepared from bovine Achilles tendon which was subjected to enzymatic processes. Changes like decreased antigenisity, control of solubility, strength and resorption can be accomplished through change of composition and structure, which makes collagen an ideal material for use as a tissue substitute (Simpson, 1983). In this study, there was no adverse reaction in any of the dogs under the study. Weissman *et al.* (1990), reported that oxygen permeability of collagen shields depend on its hydration status which directly influence corneal healing.

5.4.4. Temporary tarsorrhaphy

Temporary tarsorrhaphy was performed in eight cases except in Case No.1. As the retention of the material was not clear in the first case on first day, temporary tarsorrhaphy was employed in all other occasions. It was useful as a part of of treatment for corneal ulcers also. It did not prevent topically applied medications from reaching the eye unlike with the use of third eyelid flaps. The medication can be applied to the medial canthus and is not inhibited from reaching the ulcer and it allows some visualization of the cornea (Malenda, 2000).

5.5. EVALUATION OF THE TECHNIQUE

5.5.1. Anaesthetic protocol

. In this study, the use of atropine sulphate, xylazine hydrochloride and ketamine hydrochloride produced sufficiently deep anaesthesia for surgery of the cornea and use of 4% Lignocaine hydrochloride produced topical anaesthesia for eye examination. Analgesia was found to improve the animal's comfort and minimize the tendency for self trauma because of the rich innervations of the cornea. Excessive use of topical anaesthetics is to be avoided since it may be irritating and can cause transient conjunctival

hyperemia, systemic toxicosis and respiratory collapse (Collins *et al.*, 1995). They may be damaging to the corneal epithelium, delay corneal wound healing and mask signs when used continuously which was absent in any of the cases.

5.5.2. Suturing of the corneal wounds

In this study, treatment of such defects were done according to the principles described by Glover (2000) which involved partial thickness sutures placed perpendicular to the wound and 1 to 1.5 mm apart. In perforations, the corneal tissue can be sutured directly or the area has to be covered with a conjunctival graft or transplanted cornea. In one of the cases, (Case No. VI), the sutures disrupted due to excess tension when anterior chamber was reformed and in the other (Case No. VII,) sepsis of the eyeball was noticed which resolved following flushing the eyeball with gentamicin and further medications including antibiotics based on culture and sensitivity.

5.5.3. Application of collagen diskette

5.5.3.1. Collagen grafts in corneal healing

The collagen diskettes were completely absorbed by third postoperative day and healing was found to be progressing. Epithelialization was completed in about seven days which was reported to be influencing further healing of cornea. All the cases showed clearing of the cornea by 60^{th} day. The suitability of collagen (fibrillar matrix) as a scaffold for corneal epithelial cell infiltration and migration was proven by Orwin and Hubel (2000) which is supported by the findings in this study. Biomaterial grafts were recommended for lamellar keratoplasty in which the anterior stroma and epithelium are removed. Porcine small intestinal submucosa used by Lewin (1999) and Bussieres *et al.* (2004), was a natural biomaterial that retained the natural composition including collagen type I, III and VI. Reports describe

that the material is incorporated into corneal tissue during the healing process. Tarsorrhaphy was maintained for 16 days to prevent wound dehiscence. Fibroblastic changes were observed during histomorphological study of corneal graft healing in dog (Bussieres *et al.*, 2004).

The corneal defects will be covered by migrating epithelial cells and subsequent proliferation to restore the thickness of the epithelium. As per Startup (1984), in many cases this will not be sufficient to completely fill the defect, which may result in a corneal depression and in a few cases, a permanent epithelial pit may remain. In any of the cases studied, no such change was noticed which was attributed to the action of collagen diskette as a patch over the wound.

The amniotic membrane with large quantity of collagen acts as a substrate for epithelial growth for axonal regeneration which is having anti-scarring effect also. A rapid epithelialization occurs because the basement membrane and the avascular stromal matrix, is provided by the substance (Barros *et al.*, 2005). The biocompatibility and biodegradability of collagen plus its ready availability provided the impetus for the concept of a therapeutic collagen lens (Robin *et al.*, 1990). Compared to other methods like those employing eye patching shields provide some degree of sight (if left in the conjunctival cul-de-sac), allow release of antibiotics and do not require fitting as they are biodegradable.

The research findings were in accordance with the reports on collagen as to reduce the inflammatory and polymorphonuclear (PMN) reaction, stromal oedema and keratocyte reaction, while at the same time promoting corneal epithelial healing. Collagen shields are reported to have a therapeutic role by acting as a substrate for collagenases and PMN phagocytosis, thereby limiting corneal stromal collagen destruction as well as restoring the integrity of the corneal epithelium (Willoughby *et al.*, 2002).

5.5.3.2. Collagen as a drug delivery system

In this study, the capacity of collagen to favour healing process and act as a drug reservoir was utilized though the drug level was not measured. The natural biodegradability and high absorptive capacity of collagen makes the collagen shield an effective drug delivery system (Shaker *et al.*, 1999). When a collagen shield is used after surgery, the eye patch can be left undisturbed, while the shield releases antibiotic. There were reports on its efficacy on corneal healing also (Willoughby *et al.*, 2002). Moreover, the use of collagen can replace a painful and risky sub-conjunctival antibiotic injection (Hariprasad *et al.*, 2004).

5.5.4. Prevention of mutilation

In all the animals use of Elizabethan collar was found useful in preventing self- mutilation and it was well tolerated by the animals. It was found to be very effective in preventing self-mutilation and trauma as supported by the observations of Startup (1984).

5.6. PATIENT EVALUATION

5.6.1. General clinical condition

All the animals were in good body condition except Case No. IV. The animal in Case No. IV was very weak and debilitated, but its condition was improved on vitamin and mineral supplementation. Gum (1991) has reported that glycogen in the corneal epithelium is the energy source under stressful conditions like trauma or surgical wounds and therefore, if the glycogen stores are depleted, normal healing of epithelium and cellular locomotion over the surface is inhibited.

5.6.2. Physiological parameters

None of the cases showed any obvious signs of any systemic illness as evidenced by the examination findings. Because the ocular involvement may indicate systemic disease, a general physical examination should precede the ophthalmic examination as per Felchle and Urbanz (2001).

5.6.3. Visual function evaluation

All the visual function tests could not be conducted in all cases. Visual function tests like menace test and the pupillary reflex test were used as per Felchle and Urbanz (2001). The major drawback noticed during the study was the difficulty in getting the observations when a cornea is injured and cloudy.

5.6.4. Fluorescein dye test

Fluoresccein was used to identify the presence of a corneal ulcer or erosion in which the stromal tissue took up the dye to stain brilliant green. All the affected eyes became negative for dye retention by seventh postoperative day, which helped to demonstrate an intact corneal epithelium. Retention of fluorescein was not noticed in any of the normal eyes by Slatter (1973) and he reports that it does not enter into the cells as the other ophthalmic dyes. The same principle had been utilized in former works related to corneal healing (Bedford, 1982; Helper, 1989; Gelatt, 1991) which was due to the water soluble nature of fluorescein that does not penetrate the intact lipophilic corneal epithelium, but do stain the intercellular spaces of the corneal stroma when the corneal defects are present.

5.6.5. Corneal vascularization

Neovascularization of the cornea was observed in all cases except in II, IV and IX. Brightman *et al.*, observed the same after lamellar corneal grafting in dogs, which they attributed to the prolonged presence of suture material.

Corneal vascularity was found to be increasing and reaching the peak by day7 in all other cases except in Case No. I. The vascularisation of the cornea is an important part of corneal healing as reported by Krawitz (1963); Startup (1984); Culton *et al.* (1990) and Michau *et al.* (2003).

According to Krawitz (1963) the corneal vascularization is the result of nutritional inadequacy of cornea during healing. As the cornea is avascular, it is receiving nutrients from the tear and aqueous. Whenever there is an injury and healing process is going on this nutrient supply becomes inadequate. This stimulates growth of new blood vessels from the nearest limbal area into the cornea.

The findings of Culton *et al.* (1990) suggest that hypoxia plays an important role in corneal vascularization during healing. When the cornea faces with increased metabolic needs, but insufficient oxygen to meet these needs, the cornea undergoes anaerobic glycolysis, leading to an increased production of lactic acid. Lactic acid has been implicated as an angiogenic factor, also lactic acid and hypoxia stimulates macrophages to release angiogenic activity. There is also relationship between the inflammatory response and the growth of blood vessels into the cornea since it was inhibited by flurbiprofen, an inhibitor of prostaglandin synthesis.

5.6.6. Corneal clarity

In three cases (I, III and VI), the degree of opacity was found to increase on third day after surgery. In three case nos., (II, VII and VIII) there was improvement in corneal clarity and in case nos. IV, V and IX there was no variation in the corneal clarity on seventh day and increase in corneal opacity was noticed in Case No. IV. In all the other cases there was not much improvement.

By 14^{th} day, except in Case Nos. II and V, all eyes became clear at the

uninjured part and these two cases also regained normal clarity by 28th day. During the study, Samuelson (1991) had explained the factors essential to maintain corneal clarity and opined that any factor that adversely affect it will lead to corneal opacity, which included lack of blood vessels, non- keratinized surface epithelium maintained by preocular tear film, lack of pigmentation, partially dehydrated state and size and organization of collagen fibrils.

Since the observation was made for a maximum period of 90 days, complete clearing of the cornea could not be observed in all cases. Clearing requires more than three months. However the application of collagen diskette confirmed early healing and hence clarity was achieved in certain cases.

5.7. HAEMATOLOGICAL EVALUATION

The haematology in the affected animals did not vary much from the normal range to indicate any underlying disease process (Schalm *et al.*, 2000).

5.8. CULTURE AND SENSITIVITY OF CORNEAL SWAB

The culture and sensitivity of the corneal swab provided variable results in the cases studied. The material was collected and tested according to the method adopted by Miller and Crenshaw (1988), Massa *et al.* (1999) and Felchle and Urbanz (2001). The results revealed the presence of Gram-positive cocci, with colony characters of *Staphylococci.* The reports of Sansom (1988), Massa *et al.* (1999) and Gerding *et al.* (1993) indicated *Staphylococcus* spp. with the highest occurrence. Being the most frequently isolated conjunctival and eyelid organism, endogenous *Staphylococcus* spp. are considered as potential pathogens. The antibiotic for therapy was selected based on the culture and sensitivity result and this was found to be a very reliable method in all cases of corneal injury or ulceration or perforation.

5.8.1. Ocular therapeutics

The antibiotic of primary choice was ciprofloxacin, which was found to be effective in majority of the cases. This result was supported by the recommendations of Munro (2001), to employ frequent application of fortified solutions in deep and rapidly progressing ulcers or topical ciprofloxacin as a simpler alternative.

In most of the animals, previous medications did not make any significant difference in healing process. However, the healing rate seemed to have slowed down by continuous use of steroidal preparations as seen in Case No. III. In this case though the injury was comparatively shallow than in most of the other cases the healing was very much delayed. This finding supports the results of Martin (1971) which described the suppression of fibroblastic activity and healing by the corticosteroids. The lack of epithelium has a profound depressive effect on fibroplasias and the maturity of collagen; resulting in a marked decrease in wound tensile strength.

5.9. EXFOLIATIVE CYTOLOGY OF LACRIMAL SMEAR

The material for cytology was collected early in the examination, to reduce the chances of altering the cytological features by manipulation of the lids or globe and the examination was carried out according to the suggestions of Lavach *et al.* (1977). In this study, the polymorphonuclear cells were observed in largest number in Case No. I, which was presented soon after the occurrence of injury. This finding coincides with their report that in conjunctivitis cytologic examination was most helpful when performed early in the course of the disease. Anuclear keratinized cells were found in large numbers during the initial phase of healing of cornea especially in deeper injuries and protracted cases. The cells decreased in number, in the later phase of healing which was considered as a favourable sign in this study.

5.10. EVALUATION OF COLLAGEN DISKETTE

5.10.1. Feasibility of the technique

The collagen diskette was supposed to remain in position when soaked in antibiotic solution and applied over the corneal lesion. However, difficulty was encountered in this, as the flat diskettes were not conforming to the varying corneal curvatures in different breeds of dogs and in different conditions. The diskette had to be kept in flush with the corneal surface by removing a folded portion and without air bubbles inside. Moreover, the movement of the third eyelid in dogs made the diskette to dislodge with its powerful sweeping action on the cornea. But the application of partial temporary tarsorrhaphy was found effective in ensuring the retention of the collagen diskette.

5.10.2. Absorption of collagen

The collagen diskettes applied were not identified on third day which denotes that its absorption is more or less complete within three days. The site of lesion becomes clearer which shows the effect of collagen at the site. The corneal cells were observed to intermix with the collagen diskette, which indicates that the shields were acting as a substrate for cellular adherence and colonization (Geasey *et al.*, 1992). The concentration of the inflammatory cells was found to be increased in the post- operative period and this can cause phagocytosis of the shield. In their work, the electron microscopy revealed the irregularity of the shield edge, which was thought to be due to eyelid mechanics, cellular activity and also the ocular enzymes. This is substantiated by the fact that dissolution rate generally was higher in post- operative eyes with increased inflammatory cell counts compared with that of the normal volunteers. They also demonstrated the protective role of the shield by restricting the migration of the inflammatory cells to the incision site and that collagen shields aid in corneal healing by sequestering these cells. The results

of the present study is in accordance with all these findings as the time period for epithelialization and healing of the defect was considerably reduced than in previous reports.

As per Shaker *et al.* (1989), the shield dissolution may involve both enzymatic and mechanical action and the rate of dissolution probably depends on several host factors, including tear volume, tear enzyme concentration, degree of inflammation and blink rate.

5.10.3. Complications

5.10.3.1. Infection

In one case (Case No.VII) sepsis of the eyeball was noticed which resolved in proper antibiotic medication. It involved a penetrating injury to the cornea, wherein the chance of entry of foreign material into the anterior chamber is very high (Bedford, 1987; Felchle and Urbanz, 2001). Therefore, thorough examination of the affected eye is indicated to facilitate the removal of such objects and prevent infection due to contamination.

5.10.3.2. Mutilation

Only in two cases (IV and VII), self-mutilation was noticed. These were associated with inflammation and itching of eyelids after surgical manipulations and tarsorrhaphy. Removal of the sutures and treatment with oral medication of promethazine theoclate was sufficient in resolving the undesirable activities.

5.10.3.3. Others

5.10.3.3.1. Pigmentation of cornea

In this study corneal pigmentation was associated with the superficial vascularization which coincides with the findings of Roberts (1954)

and Bellhorn and Henkind (1966). Irritation of the cornea leads to vascularisation accompanied by inward migration of limbal melanocytes which can result in patchy pigmentary keratitis. Resorption of pigmentation can be accomplished by removal of corneal irritant and obliteration of the superficial stromal vessels, which was observed in Case No. I, which was in accordance with the findings of Grevan (2000). It was also noticed that occurrence of corneal pigmentation was the highest in brachycephalic breed of dogs. Cases with deep as well as superficial pigmentation were reported to be more refractory to treatment than cases with purely superficial involvement (Roberts, 1954).

5.10.3.3.2. Corneal opacity

There was no significant relation with the rate of healing and the duration of illness in any of the animals but there was appreciable effect on the outcome of treatment. In Case No.IV, the injury had occurred 2 weeks back but no treatment was given at that time. In this animal, the cornea became more damaged due to exposure to the environment after proptosis, and deleterious changes had already started when it was presented. In this case, cornea became thick and pigmented and did not reverse despite medications. This case proved that the period from occurrence of corneal injury to treatment is a very important factor in determining the outcome of the treatment.

The opacification of cornea due to scarring was seen associated with wound healing which, according to Krawitz (1963), was most often irreversible due to the deposition of a type of collagen fibril not characteristic of the cornea. Wilkie and Whittaker (1997), also reported that stromal repair may be accompanied by vascular ingrowths, pigment migration and regeneration of collagen in a disorganized fashion, seen clinically as a scar.

The present study revealed the effectiveness of collagen diskettes on corneal healing, which was evident from the early healing of different types of lesions and attainment of clarity. It was also clear that in any condition, early treatment resulted in better visual outcome and fewer complications.

Summary

6. SUMMARY

The efficacy of collagen diskette in healing of corneal lesions was studied in eight dogs as nine cases. Dogs presented with a history of corneal affections were examined and suitable cases were selected for the study. The cases included both fresh injuries and older lesions not amenable to medical treatment.

The clinical conditions included corneal injury, corneal ulcer and corneal perforation and staphyloma. Affected breeds included, Pug (5), German Shepherd Dog (1), Spitz (1) and Labrador Retriever (1). The incidence of corneal lesions was found to be the highest among Pugs especially among juveniles of seven months age.

All the animals selected for the study were examined for their general condition, clinical condition of the eye including nature and extend of lesion, nature of ocular discharge, vascularization *etc.* Physiological parameters of the selected animals were recorded before and after surgery during the postoperative period. Whole blood was collected for haematological examination preoperatively. Wet film and blood smear examination were also conducted.

The affected eyes were thoroughly cleaned and examined. Culture and sensitivity study was performed in all the cases, to select the suitable antibiotic for therapy. Lacrimal smears were prepared in order to evaluate the exfoliative cytology.

The affected animals were maintained under general anaesthesia during surgery. Atropine sulphate 0.045mg/ kg body weight intramuscular and Xylazine hydrochloride 2mg /kg body weight intramuscular were used as the preanaesthetics and Ketamine hydrochloride 5mg/ kg body weight

was used intramuscularly to induce general anaesthesia and was maintained with a combination of equal volumes of xylazine and ketamine intravenously to effect.

Fresh wounds on the cornea were sutured using braided silk 8/0, which was removed after two weeks. Corneal ulcers were scarified to remove the damaged tissue and the eye was irrigated with isotonic saline solution. In cases with staphyloma formation, the corneal edges were scarified and sutured after repositioning the protruded iris.

Collagen diskettes prepared from bovine Achilles tendon were used for the present study. These were supplied as collagen diskettes by the Central Leather Research Institute (CLRI), Adyar. The diskette was moistened with ciprofloxacin eye drops for about two minutes and kept over the corneal surface. To ensure the retention of the material in the eye, the eyelids were closed with temporary tarsorrhaphy using braided silk sutures. The collagen diskette was reapplied, on subsequent examination, if the diskette was found completely resorbed, and in certain cases, until completion of corneal healing.

Post operatively, Elizabethan collar was applied to all the animals to prevent self- mutilation. Antibiotic and anti-inflammatory eye drops were prescribed for topical use in all cases. Oral antibiotics were used in selected cases. Use of suitable eye drops were continued until the lesion was completely healed.

The efficacy of collagen for healing of corneal lesions was assessed by clinical and laboratory studies conducted on 3rd and 7th days, and then fortnightly during which the animals were regularly subjected to clinical examination until the cornea became fluorescein test negative and followed up to a maximum period of three months. Complications, if, any, observed during the period were also recorded.

Physiological parameters *viz.*, respiratory rate (per minute), pulse rate (per minute), rectal temperature (°C) were recorded preoperatively and during the observation period and was found to be within the normal range.

Fluorescein impregnated strips were used for assessing the depth and extend of corneal lesions. On first day all the eyes showed retention of fluorescein dye. On seventh day, except Case V and VI all others were either fluorescein test negative or with negligible level of retention and in all the cases dye retention was absent by next review on 14^{th} day.

During the later phase of healing, vascularization of cornea developed, except cases II, IV and IX. The degree of vascularization decreased gradually after attaining a peak level by 7^{th} day. The entire cornea became devoid of vascularization by the end of first month itself in most of the cases.

The haematology in the affected animals did not vary much from the normal range to indicate any underlying disease process. In this study ciprofloxacin was primarily selected as the antibiotic for topical therapy. According to the culture and sensitivity results, ciprofloxacin was selected for continued therapy in cases I, II, III, and VI, chloramphenicol in cases IV and V and gentamicin in cases VII, VIII and IX.

The material for cytology was collected early in the examination. The polymorphonuclear cells and anuclear keratinized cells were found in large numbers during the initial phase of healing of cornea especially in deeper injuries and protracted cases. Their number subsided in the later phase of healing which was considered as a favourable sign in this study.

In all the cases the collagen diskette was found to have completely dissolved by third day. There was no remnant appreciable on the corneal surface. All the animals tolerated the collagen diskette applied. No animal was reported to have any adverse reaction to the material. There was no

excess discharge or redness noticed. Complications encountered during the study included, sepsis of eye in Case VII , mutilation of sutures, corneal pigmentation in cases IV, VII and VIII and opacity in Case IV.

From the study, the following conclusions could be arrived at.

- The incidence of corneal lesions was the highest in pugs.
- Juveniles were the age group mostly affected
- Collagen diskettes used were well tolerated by all the animals and tarsorrhaphy was useful in retaining the diskettes in position.
- Collagen diskettes applied were effective in promoting healing of corneal defects. Lesions were found to heal without much delay and complications.
- Corneal culture and sensitivity were effective to guide for selecting the antibiotic for therapy
- Microbial culture indicated *Stapylococcus* spp. as the organism with the highest prevalence rate.
- Healing of corneal epithelium was found completed by seventh day, which was demonstrated by the fluorescein dye test.
- Application of collagen showed almost clearing of the cornea and attained clarity by 28^{th} day.
- Even though clarity of the cornea at the site of lesion were not achieved within the observation period, the progress in healing showed that if the treatment is continued, healing will take place by 90 to 120 days with desirable outcome.

- Pigmentation of the cornea was the major complication among Pugs affected during the healing period, which was difficult to resolve.

References

7. REFERENCES

Barros, P.S.M., Safatle, A.M.V., Godoy, C.A., Souza, M.S.B., Barrow, L.F. and Brooks, D.E. 2005. Amniotic membrane transplantation for the reconstruction of the ocular surface in three cases. *Vet. Ophthalmol.* 8: 189-192

Barros, P.S.M., Safatle, A.M.V. and Rigueiro, M. 1999. Experimental lamellar corneal graft in dogs using preserved equine pericardium. *Braz. J. Vet. Res. Anim. Sci.* 36: 138-141

Bedford, P.G.C. 1982. The diagnosis of ocular disease in the dog and cat. *Br. Vet. J.* 138: 93- 118

Bedford, P.G.C. 1987. Ocular emergencies in the dog and cat. *Br. Vet. J.* 143: 489-497

Befanis, P.J., Peiffer, R.L. and Brown, D. 1981. Endothelial repair of the canine cornea. *Am. J. Vet. Res.* 42: 590-595.

Bellhorn, R.W. and Henkind, P. 1966. Superficial pigmentary keratitis in the dog. *J. Am. Vet. Med. Ass.* 149: 173-175

Brightman, A.H., McLaughlin, S.A. and Brogdon, J.D. 1989. Autogenous lamellar corneal grafting in dogs. *J. Am. Vet. Med. Ass.* 195: 469-475

Bussieres, M., Krohne, S.G., Stiles, J. and Townsend, W.M. 2004. The use of porcine small intestinal submucosa for the repair of full thickness corneal defects in dogs, cats and horses. *Vet. Ophthalmol.* 7: 352-359

Chandrashekar, B.R., Jayadevappa, S.M., Ranganath, B.N. and Srinivas, C.L. 1997a. Experimental corneal grafting in dogs. *Indian Vet. J.* 74: 56-59

Chandrashekar, B.R., Jayadevappa, S.M., Ranganath, B.N. and Srinivas, C.L. 1997b. A histomorphological study of corneal grafts healing in dogs. *Indian Vet. J.* 74: 429- 430

Collins, B.K., Gross, M.E., Moore, C.P. and Branson, K.R. 1995. Physiologic, pharmacologic and practical considerations for anesthesia of domestic animals with eye diseases. *J. Am. Vet. Med. Ass.* 207: 220-230

Crispin, S.M. 1993. The pre- ocular tear film and conditions of the conjunctiva and cornea. *Manual of Small Animal Ophthalmology* (ed. Petersen- Jones, S.M. and Crispin, S.M.). First edition. British Small Animal Veterinary Association, Gloucestershire. pp. 137- 171

Culton, M., Chandler, D.B., Proia, A.D., Hickingbotham, D. and Klintworth, G.K. 1990. The effect of oxygen on corneal revascularization. *Invest. Ophthalmol. Vis. Sci.* 31: 1277- 1281

Felchle, L. and Urbanz, L. J. 2001. Examining the anterior segment of the eye in small animals. *Vet. Med.* 96: 792-799

Geasey, S.D., Cerro, M.D., Aquavella, J.V. and Viola, R.S. 1992. Collagen shields as a vehicle for collecting and studying migratory cells on human corneas. *Invest. Ophthalmol. Vis. Sci.* 33: 298-303

Gelatt, K.N. 1991. Ophthalmic examination and diagnostic procedures. *Veterinary Ophthalmology* (ed. Gelatt, K.N.). Second edition. Lea and Febiger, Philadelphia, pp. 195- 235

Gerding, P.A., Cormany, K., Weisigner, R. and Kakoma, I. 1993. Survey and topographic distribution of bacterial and fungal micro- organisms in eyes of clinically normal dogs. *Canine Pract.* 18: 34-38

Gilger, B.C., Hamilton, H.L., Wilkie, D.A., Woerdt, A., McLaughlin, S.A. and Whitley, R.D. 1995. Traumatic ocular proptoses in dogs and cats. 84 cases (1980- 1993). *J. Am. Vet. Med. Ass.* 206: 1186- 1190

Glover, T. 2000. Ocular emergencies. *Kirk's Current Veterinary Therapy XIII Small Animal Practice* (ed. Bonagura, J.D.). W. B. Saunder's Company, Philadelphia, pp. 1090- 1093

Grevan, V.L. 2000. Non ulcerative corneal disease. *Kirk's Current Veterinary Therapy XIII Small Animal Practice* (ed. Bonagura, J.D.). W. B. Saunder's Company, Philadelphia, pp. 1067- 1070

Gum, G. G. 1991. Physiology of the eye. *Veterinary Ophthalmology* (ed. Gelatt, K. N.). Second edition. Lea and Febiger, Philadelphia, pp. 124- 161

Hakanson, N.E. and Merideth, R.E. 1987. Conjunctival pedicle grafting in the dog and cat. *J. Am. Anim. Hosp. Ass.* 23: 641-648

Hariprasad, S.M., Mieler, W.F., Shah, G.K., Blinder, J., Apte, R.S., Holecamp, N.M., Thomas, M.A., Chi, J. and Prince, R.A. 2004. Human intraocular penetration pharmacokinetics of moxifloxacin 0.5% via topical and collagen shield routes of administration. *Trans Am. Ophthalmol. Soc.* 102: 149–158

Helper, L.C. 1989. *Magrane's Canine Ophthalmology*, Fourth edition. Lea and Febiger, USA. 297p.

Hendrix, D.V.H., Ward, D.A. and Barnhill, M.A. 2001. Effects of antibiotics on morphologic characteristics and migration of canine corneal epithelial cells in tissue culture. *Am. J. Vet. Res.* 62: 1664-1669

Kern, T.J. 1991. The canine orbit. *Veterinary Ophthalmology* (ed. Gelatt, K.N.). Second edition. Lea and Febiger, Philadelphia, pp. 239- 255

Krawitz, L. 1963. Practical anatomy and physiology of the canine eye. *J. Am. Vet. Med. Ass.* 142: 770-775

Landry M.P., Lassaline, M.E., Komaromy, A.M., Kallberg, M.E., Brooks, D.E. and Gelatt, K.N. 2004. Aggressive ulcerative keratitis in a dog. *Vet. Med.* 99: 498-506

Lavach, J.D., Thrall, M.A., Benjamin, M.M. and Severin, G.A.1977. Cytology of normal and inflamed conjunctivas in dogs and cats. *J. Am. Vet. Med. Ass.* 170: 722-727

Lewin, G.A. 1999. Repair of a full thickness corneoscleral defect in a German Shepherd Dog using porcine small intestinal submucosa. *J. Small. Anim. Pract.* 40: 340-342

Malenda, R.M.M. 2000. Ulcerative keratitis. *Kirk's Current Veterinary Therapy XIII Small Animal Practice* (ed. Bonagura, J.D.). W. B. Saunder's Company, Philadelphia, pp. 1071- 1074

Martin, C.L. 1971. Effect of vitamin-A, antibiotic, mineral oil and subconjunctival corticosteroid on corneal epithelial wound healing in the dog. *J. Am. Vet. Med. Ass.* 159:1392-1399

Massa, K.L., Murphy, C.J., Hartmann, F.A., Miller, P.E., Korsower, C.S. and Young, K.M. 1999. Usefulness of aerobic microbial culture and cytologic evaluation of corneal specimens in the diagnosis of infectious ulcerative keratitis in animals. *J. Am. Vet. Med. Ass.* 215: 1671-1674

Michau, T.M., Schwabenton, B., Davidson, M.G. and Gilger, B.C. 2003. Superficial nonhealing corneal ulcers in horses: 23 cases. *Vet. Ophthalmol.* 6: 291-297

Miller, W.W, and Crenshaw, K.L. 1988. The basics of in clinic ophthalmic examinations. *Vet. Med.* 83: 1154- 1161

Moore, C.P. 2001. Ophthalmic pharmacology. *Veterinary Pharmacology and Therapeutics* (ed. Adams, R.H). Seventh edition. Iowa State University Press, pp. 1120- 1148

Munro, E. 2001. Advances in ocular therapeutics in dogs and cats. *In Pract.* 23: 316-327

Neaderland, M.H. 1987. Healing of experimentally induced corneal ulcers in horses. *Am. J. Vet Res.* 48: 427- 430

Orwin, E.J. and Hubel, H.V. 2000. In vitro culture characteristics of corneal epithelial, endothelial and keratocyte cells in a native collagen matrix. *Tissue Engng.* 6: 307-319

Pickett, J.P. 1995. Treating persistent corneal erosions with a crosshatch keratotomy technique. *Vet. Med.* 90:561-572

Roberts, S.R. 1954. The nature of corneal pigmentation in the dog. *J. Am. Vet. Med. Ass.* 124:208-211

Robin, J.B., Keys, C.L., Kaminski, L.A. and Viana, A. G.1990. The effect of collagen shields on rabbit corneal reepithelialization after chemical debridement. *Invest. Ophthalmol. Vis. Sci.* 31: 1294- 1300

Samuelson, D.A. 1991. Ophthalmic embryology and anatomy. *Veterinary Ophthalmology* (ed. Gelatt, K.N.). Second edition. Lea and Febiger, Philadelphia, pp. 3- 123

Sansom, J. 1988. Antibacterials in the treatment of ocular infections. *J. Small. Anim. Pract.* 29: 487-499

Sansom, J. 2000. Diseases involving the anterior chamber of the dog and cat. *In Pract.* 22: 58-70

Schalm, O.W., Feldman, B.F., Zinkl, J.G. and Jain, N.C. 2000. *Veterinary Hematology.* Fifth edition. Lippincott Williams and Wilkins, Baltimore, 1344p.

Shaker, G.J., Ueda, S., LoCascio, J.A. and Aquavella, J.V. 1989. Effect of a collagen shield on cat corneal epithetlial wound healing. *Invest. Ophthalmol. Vis. Sci.* 30: 1565-1568

Shively, J.N. and Epling, G.P. 1970. Fine structure of the canine eye: Cornea. *Am. J. Vet. Res.* 31: 713-722

Simpson, R.L. 1983. Collagen as a biomaterial. *Biomaterials in Reconstructive Surgery.* (ed. Rubin, L.R.). C. V. Mosby Company, London, pp. 109- 117

Slatter, D.H. 1973. Differential vital staining of the canine cornea and conjunctiva with fluorescein – rose bengal and alcian blue. *J. Small. Anim. Pract.* 14: 291-296

Startup, F.G. 1984. Corneal ulceration in the dog. *J. Small. Anim. Pract.* 25: 737-752

Suresh, J.D. 1996. Superficial keratectomy for the management of corneal wounds in canines. M.V.Sc thesis, Kerala Agricultural University, Thrissur, 117 p.

Weissman, B.A., Brennan, N.A., Lee, D.A. and Fatt, I. 1990. Oxygen permeability of collagen shields. *Invest. Ophthalmol. Vis. Sci.* 31: 334- 338

Whitley, R.D. 1991. Canine cornea. *Veterinary Ophthalmology* (ed. Gelatt, K. N.). Second edition. Lea and Febiger, Philadelphia, pp. 307- 356

Whitley, R.D., McLaughlin, S.A. and Gilger, B.C. 1995. Update on eye disorders among purebred dogs. *Vet. Med.* 90: 574-592

Wilcock, B.P. 1993. The eye and ear. *Pathology of Domestic Animals* (eds. Jubb, K.V.F., Kennedy, P.C. and Palmer, N.). Fourth edition. Academic Press, San Diego, pp. 441-529

Wilkie, D.A. and Whittaker, C. 1997. Surgery of the cornea. *Vet. Clin. North. Am. Sm. Anim. Pract.* 27: 1097-1107

Willoughby, C.E., Batterbury, M. and Kaye, S.B. 2002. Collagen corneal shields. *Surv. Ophthalmol.* 47: 174-182

PROCESSED COLLAGEN GRAFT FOR THE TREATMENT OF CORNEAL LESIONS IN DOGS

T. A. RAJI

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2006

Department of Veterinary Surgery and Radiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

The efficacy of processed collagen diskette on corneal healing was evaluated by using the same in nine cases of corneal lesions presented to the Veterinary Hospitals of College of veterinary and Animal Sciences, Mannuthy. All the animals selected for the study were subjected to detailed clinical, haematological, wet film and blood smear examination and culture and sensitivity test of corneal swab before surgery. Exfoliative cytology and fluorescein test was conducted to evaluate healing process before and after surgery during the postoperative period.

The incidence of corneal lesions was found to be the highest among pugs especially among juveniles of seven to eight months of age. Fresh wounds were sutured using braided silk 8/0 and superficial lesions including ulcers were scarified. Corneal perforations with staphyloma were sutured after reducing the protruded portion of iris.

Collagen diskettes prepared from bovine Achilles tendon soaked in antibiotics were used for the present study. Antibiotic (selected according to the culture and sensitivity results) and anti-inflammatory eye drops were prescribed for topical use in all cases and oral antibiotics in selected cases after surgery.

The collagen diskettes could be applied very easily and were retained in position by tarsorrhaphy. The collagen applied was completely disappeared on the third day and the lesions were found healing from third day onwards. Healing of corneal injury/ ulceration/ perforation and staphyloma formation were found satisfactory and clarity of the cornea were observed from 28^{th} day onwards and the cornea became more or less clear by about 60^{th} day.

Vascularization of cornea developed in all the cases, except three, during the first week after surgery and then gradually decreased. Exfoliative cytology revealed presence of nucleated epithelial cells, anuclear keratinized cells and varying number of polymorphonuclear cells in these cases. Complications encountered during the study included, mutilation of sutures in two and corneal pigmentation in three.

Collagen diskettes used were found effective in promoting healing of corneal defects. Lesions were found to heal without much delay and the clarity of the cornea achieved was more or less complete except for a small scar at the site. All the animals tolerated the collagen diskette applied. Adverse reaction to the material was absent in any of the animals.