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RESEARCH ARTICLE

Comparison of the Efficacy of the Nictitating Membrane Flap, Conjunctival Pedicle Flap, and Platelet-Rich Fibrin Membrane Graft Techniques in the Surgical Management of Corneal Necrosis in Cats: A Retrospective Study (2016-2020)

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Abstract

The presented study was designed to compare the clinical features and the surgical outcomes of three techniques, such as the conjunctival pedicle flap (CPF), platelet-rich fibrin membrane graft (PRFMG), and nictitating membrane flap (NMF) applied after lamellar keratectomy to treat corneal sequestrum in cats. The postsurgical outcomes, complications, and recurrence rate of the lesions in 31 eyes of a total of 30 cats of different ages, breeds, and gender diagnosed with corneal necrosis were evaluated in the study. After partial keratectomy, patients were divided into three groups, each containing ten animals. Perioperative findings of all cases that underwent CPF, PRFMG, and NMF applications, including postsurgical complications, were evaluated. All patients were regularly monitored. Advantages and disadvantages were noted for all three techniques during and after the surgery. Despite long-term medical treatment with CPF (3-4 months), varying degrees of permanent fibrosis partially impairing vision developed in the related defect area, but no recurrence was observed in eyes that could not be fully keratectomy. In all cases with PRFMG, it was determined that the corneal granulation tissue disappeared completely within 5-6 weeks and the corneal transparency increased by 70% within 1.5-2 months, while recurrence developed in 2 cases. In eyes treated with NMF method, although tectonic support to the cornea and no additional tissue transplantation to accelerate the healing of the defect area was performed, the recovery period was fast and the duration of medical treatment applied to reduce or completely resolve the corneal fibrosis after surgery was very short. A rapid corneal restoration was determined in cats treated with the NMF method. Anatomical outcome was evaluated as positive in all methods. Both fast and satisfactory cosmetic appearance was achieved in PRFMG and NMF techniques. The results of this study proved that all three techniques are suitable for treating corneal necrosis in cats because of their practicality, relative effortlessness and non-invasiveness.

Keywords: *Cat, Corneal necrosis, Nictitating flap, Pedicle flap, PRF*

Kedilerde Kornea Nekrozunun Cerrahi Tedavisinde Nictitating Membran Flep, Konjonktival Pedikül Flep ve Trombositten Zengin Fibrin Membran Grefti Tekniklerinin Etkinliğinin Karşılaştırılması: Retrospektif Çalışma (2016-2020)

Öz

Sunulan çalışma, lamellar keratektomi sonrası uygulanan konjonktival pedikül flebi (CPF), trombositten zengin fibrin membran grefti (PRFMG) ve niktitans membran flebi (NMF) gibi üç tekniğin kedilerde kornea sekestrumunun tedavisinde klinik özelliklerini ve cerrahi sonuçlarını karşılaştırmak için tasarlanmıştır. Çalışmada kornea nekrozu tanısı konulan farklı yaş, cins ve cinsiyette toplam 30 kedinin 31 gözünde lezyonların cerrahi sonrası sonuçları, komplikasyonları ve nüks oranları değerlendirildi. Parsiyel keratektomiden sonra hastalar, her biri on hayvan içeren üç gruba ayrıldı. CPF, PRFMG ve NMF uygulanan tüm olguların ameliyat sonrası komplikasyonları da dahil olmak üzere perioperatif bulguları değerlendirildi. Tüm hastalar düzenli olarak izlendi. CPF ile uzun süreli medikal tedaviye rağmen (3-4 ay) ilgili defekt alanında görmeyi kısmen bozan değişen seviyede kalıcı fibrozis gelişti ancak tam keratektomi yapılamayan gözlerde dahi nüks görülmedi. PRFMG'li tüm olgularda 5-6 hafta içinde korneal granülasyon dokunun tamamen ortadan kalktığı ve 1.5-2 ay içinde korneal saydamlığın %70 arttığı belirlenirken, 2 olguda nüks gelişti. NMF yöntemi ile tedavi edilen gözlerde ise korneaya tektonik destek ve defekt alanına iyileşmeyi hızlandıracak herhangi bir ek doku nakli yapılmamasına rağmen iyileşme periyodu hızlıydı ve ameliyattan sonra kornea fibrozunun azaltılması veya tam olarak çözülmesi için uygulanan medikal tedavi süresi oldukça kısaydı. NMF yöntemi ile tedavi edilen kedilerde hızlı bir kornea restorasyonu belirlendi. Tüm yöntemlerde anatomik sonuç olumlu olarak değerlendirildi. PRFMG ve NMF tekniklerinde hem hızlı hem de tatmin edici kozmetik görünüm sağlandı. Bu çalışmanın sonuçları, pratiklikleri, göreceli zahmetsiz olmaları ve invaziv olmamaları nedeniyle kedilerde kornea nekrozunun tedavisi için her üç tekniğin de uygun olduğunu kanıtladı.

Anahtar sözcükler: *Kedi, Kornea nekrozu, Niktitans flep, Pedikül flep, PRF*

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Introduction

Corneal sequestration, also known as corneal mummification, corneal nigrum, and primary necrotizing keratitis, is a degenerative corneal disorder encountered in cats and horses, affecting all corneal layers from the epithelium to the Descemet membrane [1,2]. Although all cats are affected, Burmese, Persian, Siamese, and Himalayan cats are the most susceptible breeds reported [3].

The etiology of the disease is not fully understood. Various causes and predisposing factors, such as entropion, lagophthalmos, reduced corneal sensitivity, ulcerative keratitis, trichiasis, tear film abnormalities, genetics, feline herpesvirus-1 infection, have been reported [1,2,4,5]. Although there is no indication of age and gender-related predisposition, corneal sequestrum is most commonly seen between the ages of 2-7 years [6-8].

Corneal necrosis is a lesion located in the cornea's central or paracentral region as a unilateral, oval-to-round, black-to-brown, pigmented, and localized plaque [2]. In the standard approach to corneal necrosis, the treatment of choice is either surgery or medical therapy, depending on the lesion's size and the affected corneal layers' depth. Medical therapy is preferred when the lesion is restricted to the corneal epithelium, while the surgical approach is the best option if the lesion has advanced to the deep layers [1]. The most desired treatment in managing the Feline corneal sequestrum (FCS) targets recovering structural integrity with subtle alteration in corneal transparency. In most cases, veterinary ophthalmologists are inclined to treat the lesions either by applications providing tectonic support to the healthy cornea or a superficial keratectomy. The tectonic support might be achieved by conjunctival grafts, biomaterials, corneoscleral transposition, and keratoplasty [9]. Even though some authors proposed superficial keratectomy and NMF as a one-step combined method, it is feasible for the lesions in which less than 50% of the corneal stromal depth is involved. When the lesion affects deeper layers (50-70% of stromal depth), alternative methods such as conjunctival grafting, corneoconjunctival transposition (SKK), biomaterial grafting [using an amniotic membrane (AM), bovine pericardium, and porcine small intestinal submucosa (SIS)], and keratoplasty are required to reinforce corneal strength. More extensive lesions (>70% of stromal depth) and full-thickness FCS were previously fixed by AM grafting, SIS, and keratoplasty, which require expertise, sophisticated skills, and special equipment. Moreover, providing and storing donor tissue might be a hindrance. The relevant inconveniences necessitate an alternative practical, easily accessible, less costly, replicable, and sustainable technique, which involves an optically transparent biocompatible material with minimal health risk, yet with optimum biomechanical strength to properly suture ocular surfaces [9].

The PRFMG was efficiently and reliably utilized as a coadjuvant therapeutic agent, alternative to biomaterial grafting, to surgically treat ocular surface disorders in humans. The graft enhances tissue regeneration, minimizing inflammation and fibrosis. The fibrin membrane rich in growth factors is a standardized and optimized technology concerning tissue repair and regeneration, with highly qualified features, such as the ability to induce tissue regeneration, bactericidal and bacteriostatic activity, potential anti-inflammatory and antifibrotic properties, compatible with those of an biomaterial grafting. Multiple preclinical and clinical trials have well documented its success in human ophthalmology [10].

To the best of our knowledge, no reported data is available regarding the outcomes of the PRFMG technique in cats. Therefore, the presented retrospective study was designed, aiming to evaluate the effectiveness and outcomes of the NMF technique individually or in combination with either the CPF or a novel technique, the PRFMG, in the surgical treatment of deep corneal ulcers followed by lamellar keratectomy in cats with deep and full-thickness corneal necrosis of varying etiology. The study also aimed to provide insight into these techniques' reliability and efficacy regarding potential recurrence.

Material and Methods

Ethical Approval

This study carried out was approved by the Committee of Istanbul University-Cerrahpaşa, Animal Experiments Ethics with the decision numbered 2021/34.

Animal

The study material consisted of 30 cats of different breeds, ages, and gender diagnosed with corneal necrosis in Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Surgery between February 2016 and October 2020.

Ophthalmic Examination

All patients underwent a complete ophthalmic examination, including a series of tests such as pupillary light reflex, menace response, dazzle reflex, fluorescein staining test, Schirmer tear test, and intraocular pressure measurement, and fundus examination.

The cats suffering from corneal necrosis pertinent to the surgical intervention were elected by ophthalmological exam, yet the patients with eye conditions apt to hamper corneal healing, such as dry eye and loss to follow-up, were excluded from the study. Patients admitted for surgery were intraoperatively standardized as either deep stromal or full-thickness corneal necrosis and were randomly included in one of the three study groups, each containing ten cats.

Preparation for Surgery and Lamellar Keratectomy

Topical antibiotic ofloxacin (Exocin®, Ibrahim Abdi, Türkiye) and artificial tear drops sodium hyaluronate (Dryex®, Ibrahim Abdi, Türkiye) were applied three times a day, and ocular moisturizing gel carbomer (Thilo Tears®, Liba, Türkiye) was applied two times a day in all cases 1-7 days before the surgical procedure. Analgesia was provided with 0.1-0.2 mg/kg subcutaneous meloxicam and antibiotheraphy by 25-30 mg/kg intravenous ceftriaxone 15-20 min before surgery. Premedication with 1 mg/kg xylazine (IV) (Basilazin®, Bavet, Türkiye) was followed by induction with 5 mg/kg ketamine (IV) (Alfamine®, Atafen, Türkiye), and general anesthesia was maintained with 2-2.5% isoflurane (Forane®, Abbott, USA) and 100% oxygen. The necrotic corneal tissue was removed by cutting the stroma considering the lesion's depth using a 3.2 mm angled corneal knife. The keratectomy region was 1-2 mm larger than the corneal defect directed towards the healthy transparent cornea surrounding the necrosis *(Fig. 1-b, Fig. 5-b, Fig. 6-b, Fig. 7-b)*.

Application of Conjunctival Pedicle Flap (CPF)

After keratectomy, an incision perpendicular to the limbus was made on the eye's lateral bulbar conjunctiva. With the microsurgical scissors inserted under the incision, the conjunctive tissue was separated from the subjacent sclera by blunt dissection. The conjunctival graft's initial incision

Fig 1. Case 2: Photos showing salient steps of corneal necrosis and conjunctival pedicle flap surgery in case 2. **(a)-** Central corneal necrosis in a 2-year-old Persian cat, **(b)-** Appearance of keratectomy site and adjacent bulbar conjunctival pedicle flap of appropriate size and shape, **(c)-** The conjunctival pedicle flap is sutured over the cornea using 8-0 polyglactin 910 sutures, **(d)-** The image before cutting the conjunctival pedicle flap

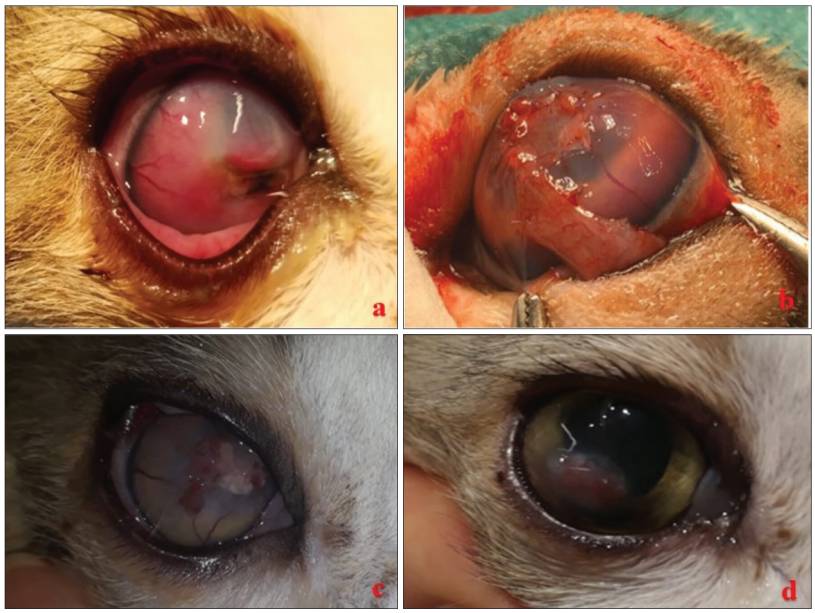

Fig 2. Case 24: After conjunctival pedicle flap surgery. **(a)-** A cat's eye with a conjunctival pedicle flap immediately after surgery, **(b)-** Edema and vascularization in flap and cornea 3 weeks after surgery, **(c,d)-** The appearance of the cornea and conjunctival pedicle flap remnants 45 and 75 days after the operation

Fig 3. Case 9: (a)- The appearance of central corneal necrosis at presentation. The remnants of the conjunctival pedicle flap 6 months after surgery. 6 weeks, **(b)-** 3 months **(c),** 4 months, **(d)-** 5 months, **(e)-** and 6 months, **(f)-** postoperative view

Fig 4. (a)- The appearance of the PRF after it is removed from the tube, **(b)-** Conversion of the PRF to the membrane form to be transplanted into the cornea

Fig 5. Case 17: (a)- Corneal necrosis concurrent with lower eyelid entropion, **(b)-** Removal of corneal necrosis by lamellar keratectomy, **(c,d,e)-** Treatment of cornea with PRFMG and NMF after lamellar keratectomy and eyelid with Holtz celcus method, **(f)-** The appearance of the cornea on the 30th day **(f),** 40th day **(g),** and 60th day **(h)**

was made with scissors parallel to and approximately 1-2 mm away from the limbus, the second incision cut was directed parallel to the initial wide enough to cover the corneal defect, and then the conjunctiva was released. The conjunctival graft's base was placed at 12 o'clock, extending vertically from the limbus to the defected area. The graft's length was determined based on the graft's distance to the site, and the graft was sutured to the

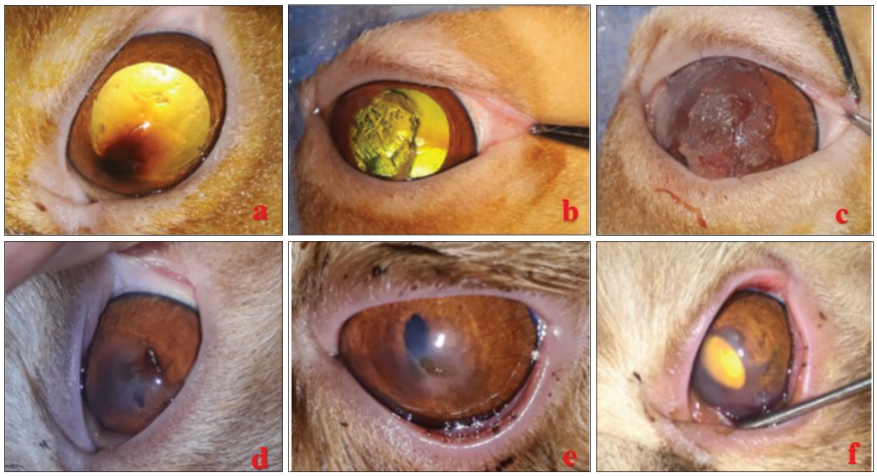

Fig 6. Case 19: Photos showing the PRFMG graft. **(a)**- Appearance of corneal necrosis, **(b)**- View of the cornea after lamellar keratectomy, **(c)**- Fixation of the PRFM graft to the cornea with 8/0 polyglactin 910. Recurrence of corneal necrosis was at the margin of keratectomy 6 **(d)** and 7 weeks **(e)** after the surgery, **(f)**- Removal of necrosis for the second time with total lamellar keratectomy and then appearance of the cornea 3 weeks after NMF application alone

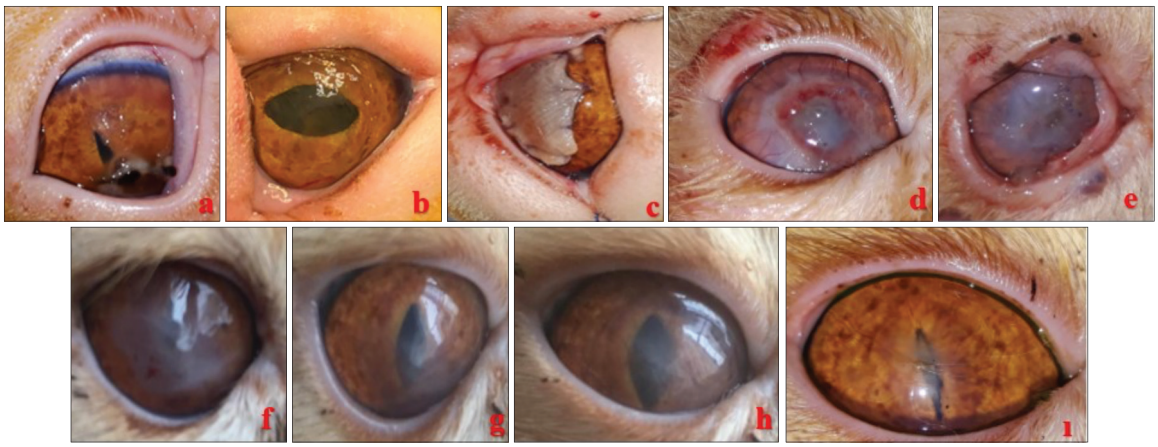

Fig 7. Case 16: (a)- Appearance of corneal necrosis, **(b)**- View of the cornea after lamellar keratectomy, **(c)**- Fixation of the PRFM graft to the cornea with 8/0 polyglactin 910, **(d)**- The dissolution of the PRFM graft in certain areas on the cornea and its replacement by marked vascularization and granulation tissue. The appearance of the cornea and PRFM graft 3 week after the surgery, **(e)**- Superficial vascularization and focal areas of pigment in the defect area and a few remaining sutures, **(f,g,h,ı)**- Clinical appearances of the cornea at intervals of 1 week.

keratectomy area on the cornea with simple interrupted sutures starting with the distal tip using an 8/0 polyglactin 910 (Vicryl®, Ethicon) suture material *(Fig. 1-c, Fig. 2-b)*.

Application of Platelet-Rich Fibrin Membrane Graft (PRFMG)

The procedure used in the study to prepare a platelet-rich fibrin membrane was modified from that previously described for human PRFMG application [11]. Three-5 mL of fresh blood was collected from the sedated cats' jugular vein into 10-mL anticoagulant-free tubes 10-15 min before the surgery. The tubes were centrifuged immediately at 2700 rpm for 12 min using a Hettich 200 EBA model centrifuge to separate platelet-poor plasma (PPP), platelet-rich fibrin (PRF), and red blood cells. The upper two layers consisting of PPP and PRF were gently removed from the tube with the aid of forceps, and the red blood cells collected at the bottom were cut with sterile scissors *(Fig. 4-a)*. The fibrin clot was converted into a membrane by being pressed gently by sterile gauze to be sutured to the corneal ulcer bed *(Fig. 4-b)*. The PRF membrane graft's length and breadth were adjusted to those of the corneal lesion, assuring that the graft was 1-2 mm larger than the corneal defect. Then, the membrane graft was sutured to the healthy corneal tissue at the lesion's margins with simple interrupted sutures using 8/0 polyglactin 910 (Vicryl®, Ethicon) *(Fig. 5-c, Fig. 6-c, Fig.7-c)*.

After the two techniques mentioned above (CPF and PRFMG) were performed, a nictitating membrane flap was applied for three weeks to protect the graft materials against drying *(Fig. 5-d,e)*.

Application of the Nictitating Membrane Flap (NMF)

The nictitating membrane flapping, a relatively simple technique, was performed to support and accelerate ulcer area healing after lamellar keratectomy. The flap was applied in a U-suture type using a 2/0 polyglycolic acid suture material and kept for three weeks.

Postoperative Management

Postoperatively, topical ofloxacin (Exocin®, Ibrahim Abdi, Türkiye) and sodium hyaluronate (Dryex®, Ibrahim Abdi,

Türkiye) drops were applied for two weeks as five times and three times a day, respectively and Elizabethan collar was prescribed for each patient during at least 4-6 weeks. The Nictitating membrane flap sutures were removed three weeks after the operation. Then carbomer-containing artificial tear gel (Thilo tears®, Liba, Türkiye) was applied as three times a day. Exocin and Dryex eye drops were continued for the following 1 week while prescribed five times a day in fluorescein stain-positive patients with incomplete corneal ulcer healing. At fourth weeks, topical ofloxacin was replaced by topical steroid eye drops (Tobramycin and dexamethasone 0.3% suspension -Tobradex®, Novartis, Türkiye) prescribed four times daily to alleviate corneal scarring.

Six to eight weeks after the surgery, the pedicle flaps were cut using corneal scissors under sedation and/or local anesthesia according to the patient's clinical status *(Fig. 3-b)*.

Results

A total of 30 cats (23 Persian, 5 British Shorthair, 1 Sphynx and 1 Domestic Shorthair cats) with corneal sequestrum were treated in the study. Fifteen were males, and fifteen were females, seven of which were spayed, and the other two were intact. The affected cats' average age was 3.5 years, ranging from 1.5 to 13 years of age. The findings regarding the breed, age, gender, clinical history, surgical methods applied, and the postsurgical outcomes of all patients were summarized in *Table 1*.

The corneal defect was caused by entropion *(Fig. 5-a)*, debridement of indolent corneal ulcer, topical steroid treatment followed by FHV-1 infection *(Fig. 2-a)*, and trauma in twelve, three, three, and other three patients, respectively. The lesion had developed due to miscellaneous causes of unknown origin in nine cats *(Fig. 1-a, Fig. 3-a)* mostly detected in Persian cats with central corneal localization *(Fig. 6-a, Fig. 7-a)*.

In the CPF technique, 3 weeks after the surgery, it was well-positioned with the neatly placed sutures, epithelialization was monitored in the conjunctival tissue's edges, and the preclinical appearance of corneal damage improved; however, the lower portion of the flap was not fully attached to the cornea. On the other hand, the conjunctival pedicle was well-vascularized with circumferential superficial corneal neovascularization, and the flap was well aligned and unnoticeable at the corneal margin *(Fig. 2-c)*. The flap was thicker and looked more hyperemic than the normal bulbar conjunctiva in all cases. Superficial neovascularization was regressed to 60% in the fourth week, 10% the sixth to eightieth weeks clinical inspections *(Fig. 1-d)*. The stromal corneal defect was filled up with the flap, but the stitches on some flap portions were loosened due to excessive stretching in one case (case no. 2, *Table 1)*. During the long-term follow-ups, neither pigmentation nor recurrence was monitored with the conjunctival pedicle flap technique. After 1.5-2 months, central corneal opacity diminished 20%. The components of the anterior segment of the bulbus oculi became distinguishable, of varying degrees, at the lesion site 2.5-3 months after the procedure *(Fig. 2-d, Fig. 3-c,d)*. Despite the long-term medical treatment (3-4 months), the conjunctivalization process was prolonged in the area of interest. Abundant fibrosis developed in large and centrally located defect areas, which partially impaired the vision. Scarring was mild, moderate *(Fig. 3-e,f)*, and severe in five, two, and other three eyes, respectively. The vision was restored despite moderate and severe corneal opacity in six of the patients. The CPF technique's success rate was 70% regarding corneal transparency, while corneal integration and healing process revealed a success rate of 90% in a one-step intervention. Moreover, the recurrence rate was 0% in the follow-ups.

In the PRFMG technique, A circumferential (360 degrees) superficial corneal neovascularization in all cases, the platelet-rich fibrin membrane on the ocular surface was found to have appeared much thinner than was observed with the CPF and even merged substantially and was replaced by granulation tissue with marked vascularization *(Fig. 5-f)*. An inflammatory reaction characterized by superficial inflammatory granulomas surrounding the stitches and granulation tissue formation were noted on the membrane graft *(Fig. 7-d)*. A complete integration of the PRFMG characterized by prominent re-epithelialization, granulation tissue formation and corneal neovascularization (50%) were quite prominent at the fourth week *(Fig. 7-e)*, fully covering up the entire ulcer area, which started to have subsided after this period. In the following 5-6 weeks, the granulation tissue formation completely disappeared during the corneal clearing phase *(Fig. 5-g)*. Corneal neovascularization was regressed 20% at six-eight weeks after surgery in all eyes *(Fig.7-f,g)*. The anterior segment had become quite distinguishable at the lesion site at the fourth week. After 1.5-2 months, central corneal opacity diminished 70%. Four eyes of four cases healed without any complications, while four eyes of the other four healed with minimal corneal vascularization and fibrosis *(Fig. 7-h)*. A mild brownish discoloration spot occurred in three eyes of three cases. A new area of necrosis developed in one case at the border of the former necrotic area (7 weeks later) *(Fig. 6-d,e,f)*, and in one other case, necrosis developed at the site of lamellar keratectomy (8 weeks later). The corneal restoration was achieved in all cases, and no visual impairment was monitored *(Fig. 5-h, Fig. 7-ı)*. After 1.5-2 months, mild corneal opacity was observed in three eyes, and full transparency in the other four eyes, while moderate brownish discoloration occurred in one eye, and a recurrence developed in two. The procedure's anatomical outcome was considered favorable regarding a satisfactory cosmetic appearance and successfully achieved visual recovery in a respectively short period. The success

Table 1. *The signalment, etiology of necrosis, summary of surgical and visual outcomes of all the patient's*

No	Breed	Age	Sex	Affected Eye	Etiology	Surgical Technique	Lamellar Keratectomy	Surgical Outcomes	Corneal Clarity
1	Persian	8 years	F	R	E	CPF	I	NR	Moderate opacity
2	Persian	2 years	F	R	U	CPF	C	NR	Severe opacity
3	British S	4 years	M	L	E	NMF	C	NR	Mild opacity
4	Sphynx	3 years	F	R	U	NMF	C	NR	Clear
5	Persian	2 years	F	R	U	NMF	I	R	Mild brownness
6	British S	1.5 years	M	R	C UI	NMF	I	NR	Mild opacity
7	Persian	1.5 years	M	R	E	NMF	C	NR	Clear
8	British S	3.5 years	M	R	T	NMF	I	NR	Clear
9	Persian	3 years	M	L	U	CPF	C	NR	Moderate opacity
10	Persian	3 years	M	R	E	CPF	C	NR	Severe opacity
11	Persian	4 years	F	R	E	CPF	C	NR	Mild corneal opacity
12	Persian	1.5 years	F	R	C	NMF	C	NR	Clear
13	Persian	5 years	F	L	U	NMF	I	R	Mild brownness
14	Persian	4.5 years	M	R	T	NMF	C	NR	Clear
15	Persian	13 years	F	R	E	PRFMG	I	R	Moderate brownness
16	Persian	6 years	F	R	U	PRFMG	C	NR	Mild corneal opacity
17	Persian	1.5 years	M	L	E	PRFMG	C	NR	Clear
18	Persian	3.5 years	F	R	In	PRFMG	C	NR	Clear
19	Persian	1.5 years	M	L	C UI	PRFMG	C	R	Moderate corneal brownness
20	Persian	5 years	M	L	U	CPF	I	NR	Mild corneal opacity
21	Persian	8 years	F	R	E	CPF	I	NR	Severe corneal opacity
22	Persian	1.5 years	F	R	In	CPF	I	NR	Mild corneal opacity
23	Persian	3 years	F	L	U	NMF	I	NR	Mild corneal opacity
24	Domestic S	1 years	F	R	T	CPF	C	NR	Mild corneal opacity
25	Persian	2 years	M	L	U	PRFMG	C	NR	Clear
26	Persian	5 years	M	L	In	PRFMG	I	R	Moderate corneal brownness
27	Persian	2 years	M	R+L	E	PRFMG	C	NR	Mild corneal opacity
28	British S	1.5 years	M	R	E	PRFMG	C	NR	Mild corneal opacity
29	Persian	3 years	M	R	E	CPF	C	NR	Mild corneal opacity
30	British S	2 years	F	L	E	PRFMG	C	NR	Clear

NR: *No recurrence,* ***R:*** *Recurrence,* ***I:*** *Insufficient,* ***C:*** *Complete,* ***E:*** *Entropion,* ***U:*** *Unknown,* ***In:*** *Infection,* ***T:*** *Trauma,* ***C UI:*** *Chronic Ulcer*

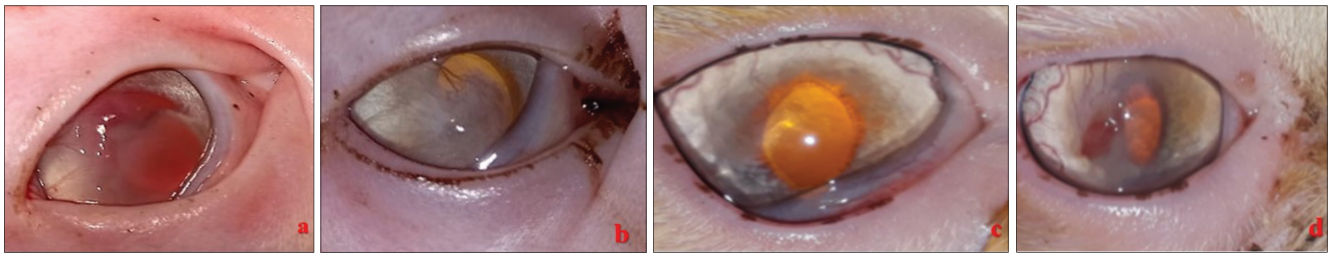

Fig 8. The appearance of the corneas 3 weeks **(a)** and 4 weeks **(b)** after membrana nictitans flap application is in case 4. The appearance of the cornea 30 days **(c)** and 45 days **(d)** after surgery in case 7

rate of the PRFMG technique was 72.7%, 81.8%, and 27.2% in terms of corneal transparency, corneal healing, and recurrence, respectively.

In the NMF technique, in the third week, the fluorescein stain-test was positive in three cases despite the well-filled defect cavity at the central cornea corresponding to the site of keratectomy and vascularization, apart from granulation tissue formation. Corneal epithelialization has not been fully completed. The anterior segment was distinguishable at the lesion site *(Fig. 8-a,b)*. Fluorescein staining was repeated at the fourth week, and corneal integrity and epithelialization were determined to have been achieved and corneal vascularization decreased to 50% at four weeks and to 20% at sixth to eight weeks of clinical examinations. The healing period was rapid, and the duration of medical therapy administered after surgery to reduce or provide full resolution of corneal fibrosis was remarkably shortened *(Fig. 8-c,d)* and even completed in 4-5-weeks in this procedure. After 1.5-2 months, central corneal opacity diminished 100%. In the cases with necrotic tissue's complete resection, no long-term complications

were monitored during 1-2-year follow-ups. Complete corneal transparency was achieved, and corneal fibrosis was indistinct. A mild corneal discoloration was noted only in two cases, which was associated with incomplete keratectomy due to the lesion's extreme depth. On the other hand, even though the necrotic tissue was unable to be excised entirely, neither the lesion recurred nor the remaining necrotic tissue fragment expanded with this technique. The NMF technique offered a less lengthy recovery period even with a deep keratectomy. The success rate of the NMF technique regarding corneal transparency was 80%. A rapid corneal restoration was determined in cats treated with the NMF method.

Discussion

In the presented study, three different techniques were utilized to repair corneal necrosis seen in cats: 1- The CPF technique, which is frequently used in the clinics, 2- The NMF technique, which is not preferable for deep lesions, and 3- The PRFMG technique, which was applied as a new technique for such lesions.

The nictitating membrane flapping is a simple, non-invasive alternative method to treat simple and spontaneous persistent corneal ulcers and corneal lacerations. The technique is also applied for postoperative fixation of corneal grafts and removal of uncomplicated corneal necrosis in cats. Its feasibility is suggested in corneal sequestrum to provide mechanical protection for the corneal surface against external trauma and eyelid friction during the corneal repair. The cornea's contact with the conjunctival mucosa's vascular network provides the release of a serous transudate that supplies the necessary substances for the cornea and also supports high temperature-responsive corneal repair processes [12]. However, unlike conjunctival pedicle flaps, NMF does not provide structural support to the cornea, including fibrovascular tissue, blood vessels, immune components, and natural anti-collegenasese. Some disadvantages arise, such as the inability to monitor the cornea during the healing process, prevention of drug penetration, the effect of pH, retention of inflammatory exudates that are in contact with the cornea, and potential surface friction. Ragozzino et al.[12] reported that the nictitating membrane flap supported the cornea, and satisfactory corneal transparency was achieved at the therapy's cessation. Neither complications nor recurrence was monitored during 1-6-year follow-ups. It was considered a successful method due to the shortened recovery time, favorable patient outcomes, and lack of recurrence [12]. However, some authors pointed out the insufficiency of the technique in deep stromal corneal defects due to the inability to perform total keratectomy in these cats, which leads to recurrence, despite the success of NMF in superficial lesions [11]. Therefore, the NMF technique was replaced by conjunctival grafting and keratoplasty targeting structural stabilization of specific lesions in the management of corneal disorders. Despite the reported disadvantages, the patients' outcomes were quite favorable with this technique. The necrosis repetition frequency in the NMF application after lamellar keratectomy was reported to have varied from 12% to 38% [12]. In the presented study, deep corneal sequestrum treated with the NMF application after keratectomy healed successfully with minimal or no scarring despite the severe stromal tissue damage even in centrally located lesions, and no vision impairment was noted. Three-year follow-ups revealed recurrence in two cats after 3-6 months. Conjunctival hyperemia and occasionally occurring blepharospasm were monitored in the other two cats, yet no recurrence was noted. The necrosis recurrence rate of the NMF application after lamellar keratectomy was estimated as 20%. Recurrence developed in two of five cases (40%) with incomplete removal. The findings were considered compatible with the previous reports, which were associated with the incomplete resection of the defected stromal tissues rather than the technique's insufficiency.

The conjunctival pedicle flapping is a simple, feasible, and cost-effective treatment method. It has long been used to treat corneal ulcers; however, its popularity has declined since new treatment options emerged. The technique aids in minimizing corneal vascularization by delivering nutrients and cellular components, and thus providing metabolic and mechanical support to the defected area, and retrieving ocular surface integrity, which -as a result- promotes wound healing and offers comfort to the patient [5,13]. It facilitates convenient monitoring of the disorder's post-operative status since it covers solely the defected area [3]. It can be easily performed in autotransplantation and donor tissue grafting with large dimensions; however, it causes the development of leucoma or scarring at the transplantation site [14]. On the other hand, the CPF technique avoids potential recurrence followed by partial excision of the necrotic stromal layer when the necrosis has invaded the deeper corneal layers. Also, it has been supported the conjunctival pedicle grafting due to its advantage as a one-step application not requiring further surgical interventions in feline corneal necrosis with persistency and potential recurrence [3,15]. In contrast, some authors suggested that it was not significantly efficient in preventing recurrence, attributing to the occurrence of persistent corneal opacity [16]. It was also indicated that the graft's overlength and flaccidity might have caused displacement due to its horizontal shifting generated by eye and eyelid movements [3]. Furthermore, insufficient debridement while preparing the corneal graft bed, the distance between the graft and the defected area, positioning the graft with an angle more than 45 degrees, the graft's inadequate size or over-thickness, excessive pressure applied on the graft, softening of the cornea, and use of improper sutures were also reported to have resulted in the conjunctival grafts' dislocation [15]. In the presented study, the pedicle flap's loosening in one patient

was associated with the ocular movements and the flap's overstretching due to its inadequacy in size. When the lesion is localized in the central cornea affecting the visual axis clarity, it is considered irrational to create transparency at the transplantation site if an optical surface is desired. It takes three weeks for conjunctivalization, and corneal transparency enters restoration at least 2.5-3 months after the operation [13,14]. Saroğlu et al.[16] reported that no vision loss was monitored with conjunctival pedicle flap, which ended up with inconspicuous, mild, moderate scarring in two, three, and two cases, respectively. In the study, in the CPF-applied patients, the lesions were centrally located in 8 cases and were paracentral in two. Five, two, and the other three patients ended up with mild, moderate, and severe scarring, respectively. Therefore, the vision was impaired to a certain degree. The recurrence rate was similar to those reported by the other researchers who performed the CPF after keratectomy. Even if there were cases where the total necrotic area was unable to be removed (n = 3), no recurrence was observed. Although it does not provide as excellent corneal transparency as other techniques and a more extended period is required to achieve corneal transparency, an improvement and vision restoration was achieved in all patients by the aid of concurrent medical therapy, which is considered a satisfactory result with an indication of the technique's feasibility.

The platelet-rich fibrin membrane is a new second-generation fibrin concentrate rich in autologous platelets developed by Choukroun et al.[17] for maxillofacial surgery. It is used in periodontal and maxillofacial plastic and reconstructive surgery and orthopedics to repair bone defects in humans [10], while it was reported to have been applied in veterinary medicine for esophageal [18] and tendon repair surgery of dogs [19,20].

The PRF membrane grafting is a successful procedure with a favorable outcome in human corneal reconstruction due to the membrane's anti-inflammatory properties and the healing process with minimal scarring. It is considered a simple, practical, and laborless technique that provides a cost-effective treatment and excellent corneal integrity [10,21]. Its accomplished application in humans inspired us to use this technique in the surgical management of feline corneal necrosis. The presented study is about the use of PRFMG material as a new method in the treatment of these lesions in cats. The fibrin matrix was transformed into a membrane graft as previously proposed by Alio et al.[10]. The membrane graft was sutured onto the intact corneal tissues while covering up the entire necrotic area. The PRFMG proved to be a successful technique applied after keratectomy to treat feline corneal sequestrum in the presented study. It was found superior to the other techniques like conjunctival pedicle flapping with minimal corneal scarring. The membrane properly integrated with the cornea, and no repositioning or correction like trimming was required. The cosmetic appearance was also quite satisfactory. Corneal transparency improved in time, and the fibrin membrane graft was replaced by corneal epithelialization. The membrane grafted provided a structural framework for full corneal recovery, and thus it is indicated in the treatment of ulcerative keratitis for supporting the corneal repair with indistinct scarring. The graft material merged with the cornea three weeks after the application, and the healing was even more satisfactory at the fourth week. Corneal thickness was within normal limits with a subtle opacity. However, it did not prevent a recurrence, particularly in cases with incomplete excision of the degenerated collagen. Recurrence developed in three of eleven eyes treated with the PRFMG technique 6-8 months after surgery, whereas no recurrence had been noted with the conjunctival pedicle flap grafting. Recurrent corneal sequestrum detected in the early postoperative period (4 weeks after surgery) occurred in two cats with incomplete excision, and vascularization during the healing process hampered distinguishing the discoloration in the defected area. Corneal discoloration recurred in one cat at the margins of the excision site despite the complete excision. It was only possible to perform an incomplete keratectomy in ten cases (3 from CPF, 3 from PRFMG, and 4 from NMF) due to the discoloration expanding into the deepest layer to avoid potential perforation. The recurrence rate was 40% (2 from PRF and 2 from NMF) in the eyes that underwent incomplete keratectomy, which was considered to be associated with the fact that residual pigmentation might have served as a potential risk factor for recurrence. The recurrence rate of the eyes that underwent incomplete keratectomy with concurrent PRFMG application was 27.2%. Nevertheless, it can be deduced that minimal scarring and rapid recovery of corneal transparency were achieved with the PRFMG technique compared with conjunctival pedicle flapping.

The study introduced some impediments to be considered. The study's retrospective design, relatively low cat population, enrolled and breed, age, and gender-wise variability were the main issues. The limited number of patients that developed recurrence hindered evaluating risk factors for recurrent corneal sequestration. Nevertheless, the study offered favorable functional and structural outcomes, with an adequate follow-up period. The conjunctival grafting followed by keratectomy reduced the potential recurrence of corneal necrosis and provided tectonic support due to a fortiori corneal scarring of varying degrees. The patients that underwent the NMF technique followed by keratectomy allowed successfully treating deep corneal necrosis, including full-thickness defects, and proved eligible for deep lesions, unlike previously described techniques. Our results revealed that the autologous PRFMG is a safe and efficient procedure in the surgical management of deep corneal necrosis and rendered merely minimal scarring compared to the CPF technique. Apart from a scaffold provided by an autologous 3-D fibrin network, the long-term release of growth

factors crucial for tissue repair, inducing the synthesis of novel collagen lamellae, enabling the emerging of the defected area smoothly into the adjacent healthy corneal tissue, is the main advantage of the technique. Briefly, the PRFMG, allowing a fifteen-minute lengthy application, was considered a well-deserved alternative to either conjunctival flapping providing tectonic support yet triggering the likelihood of axial visual impairment or other costly and more complicated methods. Even though the study was designed to investigate the efficacy of a novel technique in the surgical treatment of deep corneal necrosis, further randomized clinical trials comprising a larger patient population are required to verify the procedure's success and reveal the autologous nature of tissue repair to be offered by the technique. Consequently, the PRF membrane grafting was considered a promising treatment choice regarding the surgical approach to the patients with central and paracentral deep corneal necrosis despite the relatively low number of patients included in the presented study, which is hampering an overall inference.

Availability of Data and Materials

The authors declare that the data supporting the study findings were obtained from the corresponding author (A. Demir).

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Competing Interests

The authors declare no conflict of interest regarding this report.

Author Contributions

AD made the design of the study, the writing of the article, the treatment and controls, and the revision of the manuscript. YA provided data collection, article writing, revision of the article draft, submission of the article and entry of subsequent revisions into the system.

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