

**USE OF FIBRIN GLUE DERIVED FROM SNAKE VENOM IN THE REPAIR OF
DEEP CORNEAL ULCERS – EXPERIMENTAL STUDY IN DOGS
(*Canis familiaris*, LINNAEUS, 1758)**

**SAMPAIO R. L. (1), RANZANI J. J. T. (2), BRANDÃO C. V. S. (2), THOMAZINI-
SANTOS I. A. (3), BARRAVIERA B. (4), BARRAVIERA S. R. C. S. (5),
GIANNINI M. J. S. M. (6)**

(1) Veterinary Hospital, School of Veterinary Medicine, Uberaba University (UNIUBE), Uberaba, Minas Gerais State, Brazil; (2) Department of Veterinary Surgery, School of Veterinary Medicine and Animal Husbandry (FMVZ), São Paulo State University (UNESP), Botucatu, São Paulo State, Brazil; (3) Laboratory of Hemostasis, Blood Center, Botucatu Medical School (FMB), UNESP, Botucatu, São Paulo State, Brazil; (4) Center for the Study of Venoms and Venomous Animals (CEVAP), UNESP, Botucatu, São Paulo State, Brazil; (5) Department of Dermatology and Radiotherapy (FMB), UNESP, Botucatu, São Paulo State, Brazil; (6) Department of Clinical Analysis, Araraquara School of Pharmaceutical Sciences (FCFAr), UNESP, Araraquara, São Paulo State, Brazil.

ABSTRACT: Fibrin glue has been researched as an alternative method for tissue synthesis and is known for its capability to promote hemostasis at the application site, good approximation of wound edges and fast healing. The current study consisted in the application of fibrin glue derived from snake venom as treatment for experimental corneal ulcers. Twenty-one dogs had their corneas experimentally prepared through lamellar keratectomy (of standardized diameter and depth). Animals were divided into seven groups of three animals each. Six experimental groups were periodically evaluated and collection was carried out on the 1st, 3rd, 7th, 15th, 30th and 60th post-operative days, whereas one control group was evaluated throughout the experiment. Analyses consisted in the clinical evolution and in the histopathological study of all operated on eyes. Results indicated that fibrin glue was efficient in repairing keratectomy wounds in dogs and contributed to an earlier healing phenomenon, avoiding edema formation and keeping corneal clearness. The use of fibrin glue derived from snake venom showed to be easy to apply, feasible with animal models and of low cost, avoiding the lesion progress and allowing fast and appropriate corneal healing.

KEY WORDS: fibrin glue, snake venom, cornea, corneal ulcer, keratoplasty.

CORRESPONDENCE TO:

RENATO LINHARES SAMPAIO, Avenida Afrânio Azevedo, 2140, Olinda, 38020-450, Uberaba, Minas Gerais, Brasil. Email: renato.sampaio@uniube.br.

INTRODUCTION

Due to its anatomical site, the cornea frequently undergoes external traumas, as well as eyelid, eyelash and lachrymal apparatus abnormalities. The latter often lead to abrasions, lacerations and corneal ulcers, which interfere in clearness, endangering the vision. Thus, the severity of corneal lesions and their repair mechanisms during several morbid processes make necessary the fast institution of effective therapeutic measures. These are employed with the aim of avoiding the lesion progress, allowing a fast and appropriate healing with minimal scar formation, which can be achieved with clinical and surgical procedures. The institution of one or another treatment depends on the right diagnosis of the primary cause and on the analysis of secondary agents, besides the problem prognosis (18, 19, 35, 36).

Therefore, several researchers have studied the mechanisms of corneal healing in order to develop appropriate methods to treat corneal lesions, restoring its clearness and contributing to visual rehabilitation. Among the already investigated techniques, the most important are corneal transplants, sutures, corneal covering through the eyelids and nictitating membrane, free autogenous and pediculate grafts of the conjunctiva, and keratoplasty using biological membranes and adhesive tissue (1, 23, 31).

Adhesive application in living tissues has been tested for many years by several researchers with the aim of developing a method that could provide fast and efficient tissue synthesis, with minimal granulomatous reaction in the wound. Tarlov (1940) and Young (1944), both cited by Morandini and Ortiz (29), first tested the use of adhesive substances in living organisms. These authors used, respectively, plasma enriched with heterologous and homologous fibrinogen in the production of biological glues. Their studies are important for the historical initiative, as the results did not prove the initial hypotheses but stimulated the investigation of more efficient formulas for tissue synthesis.

From there on, the development and application of synthetical adhesives started to call the researchers' attention. Thus, several substances, such as mixture of casein and polyvinyl alcohol, derived from rubber, polyacrylate, epoxy resin, formaldehyde resin and cyanoacrylate adhesive, were tested; however, they showed to have irritating action on ocular tissues with no effective contribution to healing (6, 9, 12, 26, 27, 31).

As an alternative to corneal wound repair, biological glues based on fibrin started to be investigated and have been used as hemostatic agents (22) as well as in tissue synthesis in several surgical procedures (4, 7, 8, 22, 25, 33, 34, 40).

The biological principles of fibrin glue are similar to those of the coagulation cascade, with final formation of a fibrin clot. Several formulas have already been developed and their differences range from the fibrinogen origin and the thrombin source to the concentration of several compounds. The adhesive is prepared by the combination of a concentrate of fibrinogen and thrombin, reconstituted in calcium hydrochloride solution. The principle of adhesiveness is the strong affinity of the mixture for collagen at the moment the compounds reaction tends to imitate the last stage of the coagulation cascade, through fibrinogen and factor XIII activation by thrombin in the presence of calcium ions. Fibrinogen is then converted into fibrin, originating a stable clot strengthened by the activation of factor XIII, which also participates in collagen synthesis, stimulating fibroblast proliferation and contributing to tissue healing (7, 10, 13, 20, 38).

Fibrinolysis is essential to a successful fibrin glue application as it is necessary to the glue complete absorption. Fibrinolysis period probably depends on the plasminogen activity, which is higher in highly-vascularized nerves and tissues, inducing a fast removal of the fibrin adhesive (3, 7, 30, 39).

In ophthalmology, tissue fibrin adhesives have been little explored considering the diverse range of situations in which they can be applied, such as in the treatment of corneal ulcers and wounds, and in the replacement of suture lines during the ocular tunics synthesis. Gestring & Lerner (14) reported successful use of fibrin adhesive in experimental closure of corneal perforation. They did not mention any secondary complication due to the product application or any opacity at the application site.

Following the same principles, Lagoutte *et al.* (24) tested the fibrin adhesive Tisseel[®] in perforated and pre-perforated corneal lesions in human patients considering that this glue can be physiologically degraded, contributing to the site healing and surpassing cyanoacrylates with regard to these aspects.

Recently, Bonati *et al.* (5) succeeded in using experimental fibrin glue after perforating trephination and removal of a 3mm-diameter tissue area from the cornea of dogs. The study demonstrated a good absorption of the adhesive applied to the cornea, efficiently obstructing the perforated areas of 7 out of 10 operated on

animals, which did not show any type of iris adherence on the application region, neither signs of vascular neogenesis.

Treatment of corneal perforations was again discussed by Bonati *et al.* (3), who cited, among other methods, the application of fibrin adhesive, emphasizing its physicochemical and biological properties as well as its indication in ophthalmology. They also cited the successful clinical use of fibrin glue in two patients with corneal perforation.

In another experimental study, Bonati *et al.* (4) tested the resistance of intraocular pressure and microscopic characteristics of corneal perforations treated with fibrin adhesive. The minimum pressure was observed within 2 hours, with rupture of the glued region under pressures above 11mmHg. The maximum pressure reached 480mmHg, the measuring limit of the equipment. The histopathologic test indicated epithelization on the area in the third post-operative day, showing a progressive replacement of the fibrin glue for a scar.

Studies replacing the traditional sutures by fibrin adhesive for the closure of scleral incisions in patients subjected to the phacoemulsification technique were carried out by some authors. The final observations did not show any local complication during wound healing and the results suggested that the post-operative evolution when using fibrin glue prevented the development of astigmatism in a large number of patients (15, 16, 21, 28).

Recently, studies have shown the efficiency of fibrin adhesive in the fixation of biological membranes and conjunctiva grafts in the treatment of lesions at the ocular surface. Hick *et al.* (17) tested the fixation of the amniotic membrane with fibrinoid adhesive in 33 eyes with corneal lesions, 14 of which were classified as perforated ulcers. The authors concluded that the association of the amniotic membrane with the fibrin glue was efficient in closing perforations up to 3mm and that this technique contributed to the fast healing of the ocular surface, providing a good functional recovery of the eyes subjected to the employed keratoplasty technique.

Based on the same principles, Szurman *et al.* (37) carried out a comparative study between keratoplasty with amniotic membrane fixed with fibrin adhesive and keratoplasty fixed with 10-0 nylon suture, using rabbits as experimental model. Results indicated that the use of adhesive reduced the operation period and allowed the permanence of the membrane until the complete epithelization of the

keratectomized area. This proved the efficiency of fixation technique using adhesive, which showed to be biocompatible and did not present an inflammatory response as intense as that observed in corneas treated with suture.

The present work aimed at elucidating the mechanisms related to the repair of deep corneal ulcers treated with fibrin adhesive derived from buffalo fibrinogen and other compounds obtained from snake venom as a replacement for thrombin.

MATERIALS AND METHODS

In the present study, 21 healthy dogs were randomly divided into seven groups of three animals each. Six groups received the application of biological adhesive and one group remained as control throughout the experimental period. Then, the post-operative periods for cornea collection and morphologic evaluation were established as follows: Group 1 (24h), Group 2 (72h), Group 3 (7 days), Group 4 (15 days), Group 5 (30 days), Group 6 (60 days), Group 7 (control). The eyes were subject to antiseptis with povidone iodine diluted 1:50 in physiological solution. Using a surgical microscope, a 5mm-diameter trepan, not invasive, was applied in the central region of the right cornea to delimit the area. A corneal fragment was removed by using double-bladed scalpels and corneal scissors; the depth of keratectomy was monitored by corneal pachymeter until the thickness of the operated on area reached 200 μ m. The keratectomized areas in Groups 1, 2, 3, 4, 5 and 6 were filled with fibrin adhesive using a 1ml disposable syringe coupled to an insulin hypodermic needle. Four drops of adhesive were applied on the keratectomized area; this amount was enough for total filling. Animals of Group 7 underwent the same surgical procedure, however, without adhesive application. All groups were periodically evaluated at 24h intervals until the end of the experiment, and the following parameters were analyzed: pain, represented by signs of photophobia and blepharospasm; ocular secretion; conjunctival hyperemia; corneal edema; neovascularization; and pigmentation. All these changes were qualified and later subjectively quantified: 0 – absent; 1 – light; 2 – moderate; and 3 – intense. After the clinical test, the eyes were subjected to fluorescein test for evaluation of the epithelization degree of the ulcer treated with fibrinoid adhesive and investigation of the adherence and permanence time of the studied material on the lesions. Then, after sacrifice of the animals and collection of the eyes, cornea histological

preparation was stained using the hematoxylin and eosin technique. Slides of histological sections were evaluated under microscope, and the parameters related to congestion, edema, hemorrhage, vascular neogenesis, fibrosis, and polymorphonuclear and mononuclear leukocytes infiltration were evaluated. The same criterion was adopted for the morphological study.

RESULTS AND DISCUSSION

The adhesive showed a good capacity of fixation to the keratectomized area, forming a stable and slightly opaque clot after application (Figure 1). However, the clot of fibrin adhesive detached approximately 48h after application and, then, remained in the form of some fillets (only visible with the aid of a microscope) in three animals. The early elimination of the biological adhesive, whose main compound is represented by the fibrin clot, is due to the presence of substances in the tear that activate the little amount of plasminogen found in the adhesive, converting it into plasmin and causing lysis of fibrin into soluble products. To overcome this problem, Bonati *et al.* (2) recommended the use of tranexamic acid associated with fibrinogen, which delayed the plasmin fibrinolytic activity, contributing to the adhesive fixation for a longer period. Epsilon-aminocaproic acid has also been used in association with fibrinoid adhesives in order to decrease the tissue fibrinolytic activity and increase the permanence of the fibrin adhesive on the wound (30).

Although fibrinolysis occurred in the first 48h, healing of the eyes treated with fibrin adhesive had no complications, compared with the control group. All operated on eyes showed to be painful in the first three days of evaluation, presenting photophobia and secretion which were not due to the adhesive application but to the surgery itself, since pain was noticed in three animals of the control group that underwent the same surgical procedure without, however, receiving the adhesive (Figures 2 and 3). Discomfort in the immediate post-operative period has also been described by other authors who used the keratectomy technique as an experimental model to investigate the mechanisms of corneal healing (1, 5, 17). This fact can be explained by the stimulation of pain receptors present in the epithelium, proceeding from the ramifications of the long eyelid nerves, which start at the ophthalmic branch of the trigeminal nerve and activate the afferent branch of the corneal reflex, through

stimulation of the axons of epithelial terminations, causing blepharospasm and higher sensitivity when exposed to light (11, 32, 35).

Signs of discomfort had already disappeared on the seventh day, when blepharospasm and photophobia were not observed anymore, indicating that the stimulation of pain receptors ceased (Figures 4 and 7). Recent studies using fibrin adhesive as a substitute for the traditional sutures in the fixation of biological membranes also indicate that pain in these cases is slight and elusive, disappearing within the first hours after application, which indicates that the nervous stimulation is minimized with the use of adhesive and that terminations are efficiently protected by the membrane (17, 37).

The predominant secretion was mucous, and purulent secretion was not observed in the operated on eyes. Quantitative analysis revealed moderate manifestation in the first four days, which decreased to lower values until the 13th post-operative day, when secretion production in the eyes of all animals ceased, indicating that inflammation caused by the surgical aggression due to keratectomy disappeared (Figures 5 and 7). Mucous secretion is produced by calyciform cells present at the conjunctiva and the increase in its production can be related to aggression to the ocular surface. In this experiment, increase in mucous secretion was also related only to traumatism caused in the cornea during keratectomy, since both the adhesive-treated group and the control group presented similar levels. This response to aggression showed that the lesion present in the cornea also affected the conjunctiva, which indicates the existence of an integrated mechanism of response to diseases that attack structures present at the ocular surface; such fact was also reported for animals subjected to keratoplasty (10, 27).

Conjunctival hyperemia and chemosis were also analyzed as indicators of the intensity of inflammatory response at the ocular surface and, in this experiment, both in adhesive-treated animals and in control animals, these clinical characteristics showed low levels until the fifth post-operative day (Figure 3). After this period, they totally retrograded, indicating there was not persistence of aggressive stimulus to the ocular surface (Figure 7). Conjunctival hyperemia and chemosis are good parameters to evaluate the aggression index at the ocular surface, since these characteristics are related to the increase in vascular permeability and blood flow in sites where there is release of vasoactive factors, common during inflammation. The

presence of such characteristics in the first days indicates there was cellular aggression followed by local inflammation due to keratectomy. On the other hand, disappearance of these characteristics after the fifth day in the treated and in the control groups indicated the aggression to the ocular surface did not persist, showing that the tested adhesive, as well as its byproducts released during degradation, did not act as irritating agents to the eye (Figure 4). The satisfactory results presented by Bonati *et al.* (2) reinforced the hypothesis that the ocular tissues present good tolerance when treated with fibrin biological adhesives. These findings were also reported in subsequent works carried out by Bonati *et al.* (3), who compared the treatment of perforated corneal ulcers with other methods and concluded that the application of fibrin adhesive was efficient in the closure of corneal perforations, highlighting its physicochemical and biological properties, besides its safe use in ophthalmology.

The clinical test did not show edema in the periphery of lesions in animals subjected to keratoplasty using fibrin glue, which indicates that the adhesive acted as an impermeable material, avoiding water penetration and consequent stroma hydration. This did not occur in control animals, which showed little edema in the wound edge since the 1st post-operative day, indicating there was water penetration into the subjacent stroma of the cornea of these animals (Figures 2 and 3). Edema is directly associated with decreased clearness, and its absence indicates that the adhesive was efficient in maintaining the cornea hydric balance until repair. These were not observed in studies using synthetical adhesives or sutured biological membranes, as the former leads to release of irritating substances during degradation, limiting the migration of the anterior epithelium to the keratectomized area and allowing a slit on the wound edge during the adhesive permanence, which allows water penetration to the adjacent stroma. Membranes also suffer retraction after application, mainly at the sites where sutures penetrate, allowing the passage of tears and stroma hydration (1, 31, 37).

Epithelization of the keratectomized area was complete at three post-operative days in all adhesive-treated animals, and from this time on, impregnation of the experimental ulcer with fluorescein was not observed, indicating that there was complete epithelial covering of the corneas that received the fibrin biological adhesive (Figures 3 and 4). This indicates that the adhesive helped in the site repair,

contributing to earlier healing mechanisms, since in the control group, fluorescein fixation was observed 96h after the beginning of the experiment in two out of three animals. This corroborates the results obtained by other authors, who credit to the fibrinoid adhesive the capability to allow the migration of periphery epithelial cells over the lesion, serving as a bridge in the first hours of the repair process and contributing to the stimulation of fibroblasts migration to the wound, which accelerates collagen production and the consolidation of events related to tissue repair (2, 10, 17).

Although the initial lesion was deep and had large superficial area, the fast epithelization of the keratectomized area, with no signs of severe inflammation, contributed to a healing process without vascularization of the cornea during the observation period (Figures 5 and 6). The presence of vases is an important parameter that indicates the lesion severity, common when a great loss of tissue occurs or even in situations in which the repair mechanisms find obstacles such as contamination or destruction of limbal cells. Absence of vascularization and discrete signs of discomfort indicate there was good tolerance by the ocular surface to the adhesive, attesting its innocuousness at the application site. Pigmentation was not observed in the corneas that received the fibrin biological adhesive, as there was a slight inflammation restricted to the first post-operative days; there was not adequate stimulation for vascular formation and pigments deposition in the cornea (Figure 6).

Results of the histological evaluation met the clinical findings, showing other important details to understand the effects of biological adhesive on cornea healing. Different parameters relative to edema formation were evaluated, including vascular neogenesis, inflammatory infiltrate cells, fibrosis, and cell arrangement.

Twenty-four hours after the adhesive application, little edema was noticed in the corneas of the treated group. This phenomenon showed the same patterns during the first seven days, indicating there was slight hydration of the stroma and consequently disarrangement of the stromal collagen, which clinically manifested as higher opacity. Still in the first day, there was discrete epithelial disarrangement in adhesive-treated eyes, with a small number of neutrophils and hyperplasia of cylindrical cells. Three days after surgery, neutrophils and epithelial disarrangement with little fibrosis were noticed. Lymphocytes and plasmocytes were not observed.

After a post-operative week, slight edema was observed, along with atrophy of the anterior epithelium, little vascularization in the periphery of the cornea, fibrosis and little inflammatory infiltrate cells, composed of polymorphonuclear cells.

At 14 days, inflammatory infiltrate was discrete and still represented by polymorphonuclear cells; however, in a smaller number, relative to the previous evaluation. Moderate fibrosis and atrophy of the anterior epithelium were also observed.

After one month, the corneas that received the adhesive showed hyperplasia of cylindrical cells and a small number of neutrophils around the scar.

The corneas collected at 60 days presented very discrete alteration when compared with the material collected at 30 post-operative days. Inflammatory cells were rare, like all the other phenomena related to the healing process. Fibrosis was moderate, manifesting clinically as a slightly opaque circular area at the site where lamellar keratectomy was done for the preparation of the experimental ulcer.

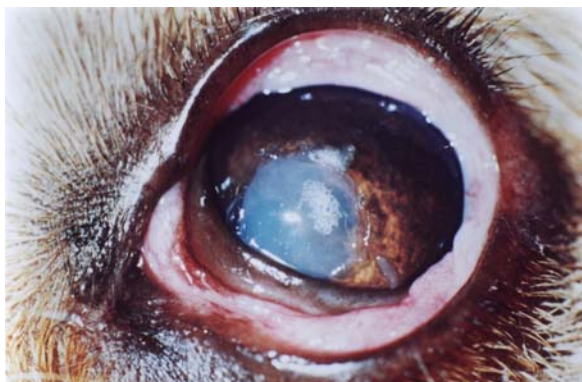


Figure 1. Detail of the keratectomized area immediately after application of the fibrin adhesive derived from snake venom in an animal of Group 1. There is a fibrin clot covering the area.

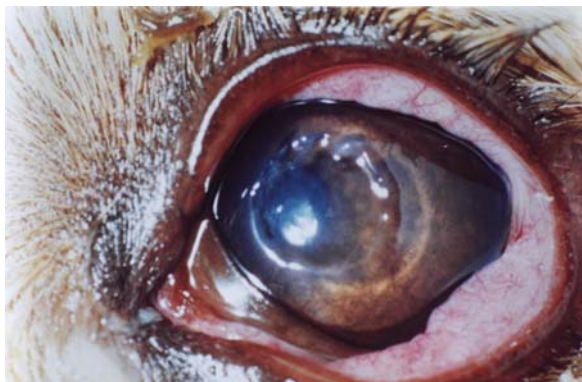


Figure 2. Detail of the keratectomized area 48h after application of the fibrin adhesive derived from snake venom in an animal of Group 2. There are fibrin adhesive residues on the keratocomized area, which does not show edema and is in an advanced stage of reepithelization.

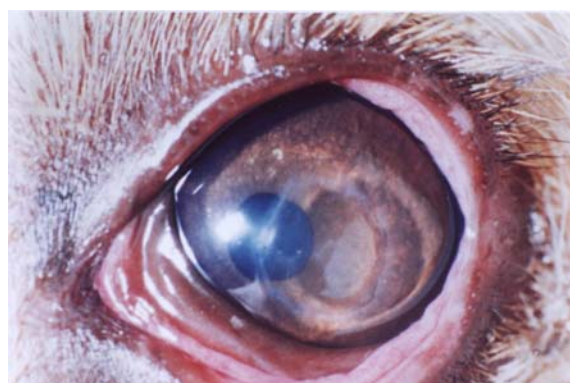


Figure 3. Detail of the keratectomized area 96h after application of the fibrin adhesive derived from snake venom in an animal of Group 3. Detachment of the fibrin adhesive had already occurred; however, fixation of the fluorescein in the keratectomized area is not observed, suggesting there was complete epithelization. Clearness in the central region of the lesion is noticed, indicating that healing occurred without formation of edema in the wound. There is also a slight area of opacity in the periphery of the lesion, representing the initial formation of a scar to perfectly repair the corneal stroma.

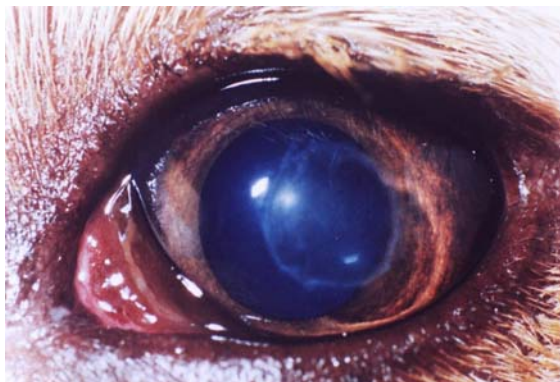


Figure 4. Detail of a dog cornea seven days after application of the fibrin adhesive derived from snake venom in an animal of Group 3. There is an increase in opacity at the periphery of the keratectomized area, indicating the progress of a scar formation.

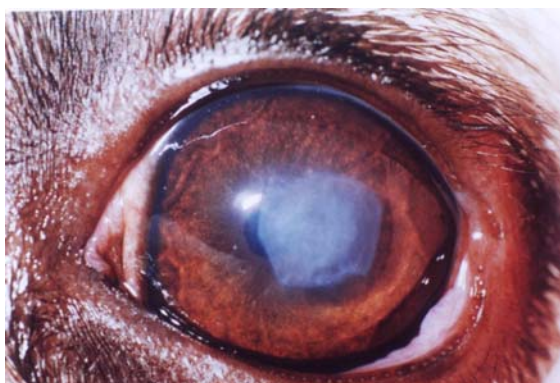


Figure 5. Detail of a dog cornea 15 days after application of the fibrin adhesive derived from snake venom in an animal of Group 4. There was total repair of the wound and complete filling of the keratectomized area with scar tissue.

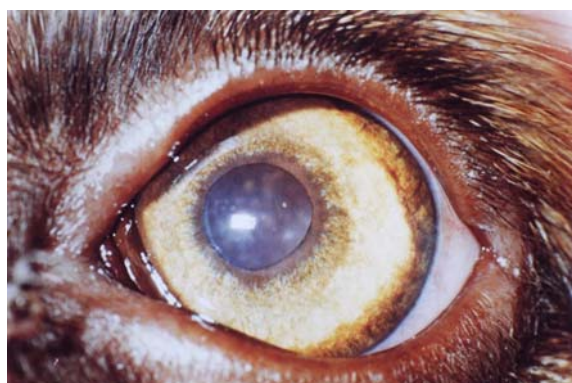


Figure 6. Detail of a dog cornea 30 days after application of the fibrin adhesive derived from snake venom in an animal of Group 5. A good recovery of clearness was observed, indicating that the repair process keeps active, promoting the organization of the corneal stroma and the visual rehabilitation of the patient.

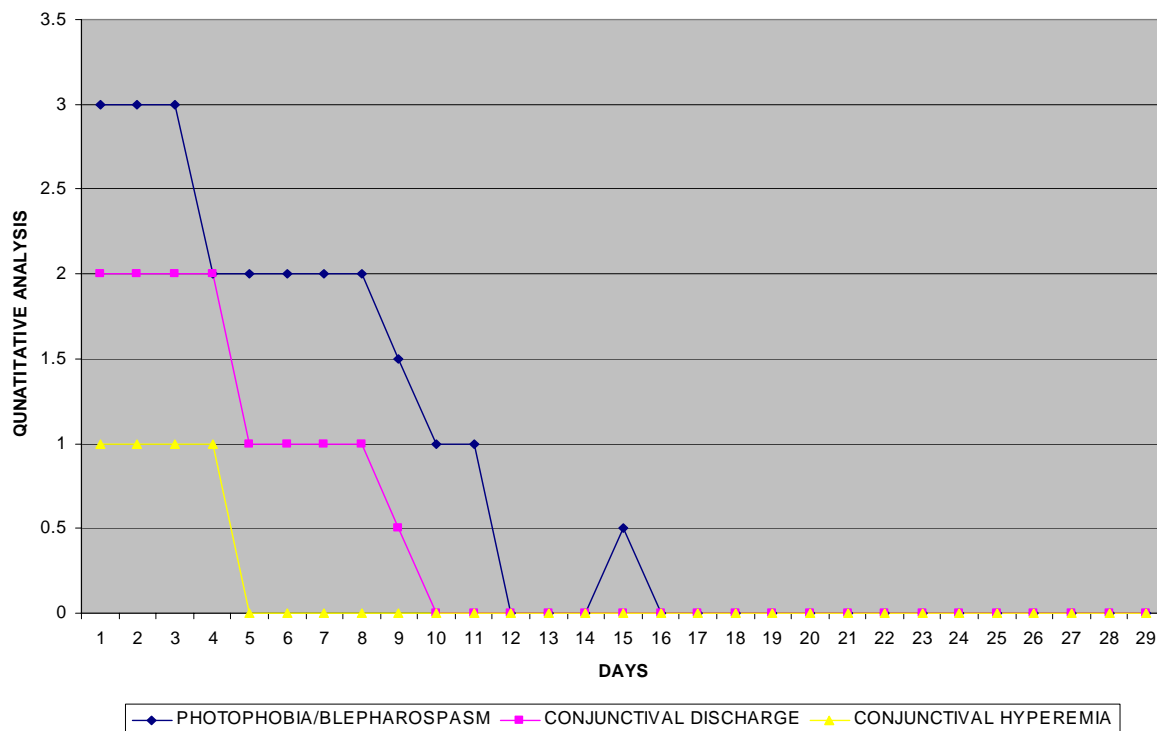


Figure 7. Graphic representation of the clinical evolution of corneas treated with keratoplasty using fibrin adhesive.

CONCLUSIONS

The obtained results indicate that the application of fibrinoid adhesives for the preparation of keratectomies in dogs is simple to apply, feasible in animal models and of low cost.

Despite the little time of permanence, the adhesive showed to be efficient, contributing to earlier reparative mechanisms, accelerating the healing process and avoiding edema formation in the wound, contributing to the maintenance of the corneal clearness

The treated eyes followed normal patterns of scar repair and, according to the present results, the biological adhesive is innocuous to tissues of the ocular surface.

The profile of the phenomena associated with discomfort and pain constitute symptoms related to the surgical procedure, which were not worsen by the application of the tested biological adhesive.

REFERENCES

- 1 BARROS PSM., SAFATLE AMV., MALERBA TA., BURNIER JR M. The surgical repair of the cornea of the dog using pericardium as a keratoprosthesis. *Braz. J. Vet. Res. Anim Sci.*, 1995, 32, 251-5.
- 2 BONATI JA., STEFANO JT., MATHEUS LCA., OLIVEIRA GA., SUZUKI H., KARA-JOSÉ N. Desenvolvimento de adesivo tecidual fibrínico para uso experimental em perfurações corneanas. *Arq. Bras. Oftalmol.*, 1995a, 58, 354-6.
- 3 BONATI JA., SUZUKI H., KARA-JOSÉ N. Tratamento da úlcera corneana perfurada: revisão da literatura. *Arq. Bras. Oftalmol.*, 1997, 60, 60-6.
- 4 BONATI JA., SUZUKI H., KARA-JOSÉ N., ALVES MR., MATHEUS LCA., ZANOTTO A., LEITÃO R. Resistência à perfuração intra-ocular e aspectos histopatológicos em ferimentos perfurantes corneanos colados com fibrina – estudo experimental. *Arq. Bras. Oftalmol.*, 1998, 61, 15-24.
- 5 BONATI JA., TOLOSA EMC., MATHEUS LCA., LEITÃO R., SUZUKI H., KARA-JOSÉ N. Cola de fibrina em perfuração experimental em cães. *Arq. Bras. Oftalmol.*, 1995b, 58, 88-92.
- 6 BORUCHOFF SA., REFOJO M., SLANSKY HH., WEBSTER RG., FREEMAN MI., DOHLMAN CH. Clinical applications of adhesives in corneal surgery. *Trans. Am. Acad. Ophthalmol. Otolaryngol.*, 1969, 73, 499-505.
- 7 BRENNAN M. Fibrin glue. *Blood Rev.*, 1991, 5, 240-4.
- 8 BYRNE DJ., HARDY J., WOOD RA., MCINTOSH R., CUSCHIERI A. Effect of fibrin glues on the mechanical properties of healing wounds. *Br. J. Surg.*, 1991, 78, 841-3.
- 9 DELLEVIGNE W., WOLFERTH JR CC., JONES N., MATSUMOTO T. Cyanoacrylate monomers as an adhesive. *Arch. Surg.*, 1971, 102, 493-5.
- 10 DUCHENESE B., TAHI H., GALAND A. Use of human fibrin glue and amniotic membrane transplant in corneal perforation. *Cornea*, 2001, 20, 230-2.
- 11 DYCE KM., SACK WO., WENSING CJG. Os órgãos dos sentidos. In: DYCE KM., SACK WO., WENSING CJG. Eds. *Tratado de anatomia veterinária*. Rio de Janeiro: Guanabara Koogan, 1990: 225-35.
- 12 EHREHAUS M., D'ARIENZO P. Improved technique for temporary tarsorrhaphy with a new cyanoacrylate gel. *Arch. Ophthalmol.*, 2003, 121, 1336-8.

13 ERDEY RA., LINDAHL KJ., TEMNYCKY GO., AQUAVELLA JV. Techniques for application of tissue adhesive for corneal perforations. *Ophthalmic. Surg.*, 1991, 22, 352-4.

14 GESTRING GF., LERNER R. Autologous fibrinogen for tissue-adhesion, hemostasis and embolization. *Vasc. Surg.*, 1983, 17, 294-304.

15 GREWING R., MESTER U. Radial suture stabilized by fibrin glue to correct preoperative against the rule astigmatism during cataract surgery. *Ophthalmic. Surg.*, 1994, 25, 446-8.

16 HENRICK A., KALPAKIAN B., GASTER RN., VANLEY C. Organic tissue glue in closure of cataract incisions in rabbit eyes. *J. Cataract. Refract. Surg.*, 1991, 17, 551-5.

17 HICK S., DEMERS PE., BRUNETTE I., LA C., MABON M., DUCHESNE B. Amniotic membrane transplantation and fibrin glue in the management of corneal ulcers and perforations: a review of 33 cases. *Cornea*, 2005, 24, 369-77.

18 HIRST LW., SMIDDY WE., DEJUAN E. Tissue adhesive therapy for corneal perforations. *Aust. J. Ophthalmol.*, 1983, 11, 202-6.

19 KERN TJ. Ulcerative keratitis. *Vet. Clin. North Am. Small Anim. Pract.*, 1990, 20, 643-66.

20 KHADEM J., TRUONG T., ERNEST JT. Photodynamic biologic tissue glue. *Cornea*, 1994, 13, 406-10.

21 KIM JC., BASSAGE SD., KEMPSKI MH., DEL CERRO M., PARK SB., AQUARELLA JV. Evaluation of tissue adhesives in closure of scleral tunnel incisions. *J. Cataract Refract. Surg.*, 1995, 21, 320-5.

22 KRAM HB., NATHAN RC., STAFFORD FJ., FLEMING AW., SHOEMAKER WC. Fibrin glue achieves hemostasis in patients with coagulation disorders. *Arch. Surg.*, 1989, 124, 385-7.

23 LAFORGE H. Diagnóstico y tratamiento de las úlceras corneales. *Waltham Int. Focus*, 1993, 3, 2-8.

24 LAGOUTTE FM., GAUTHIER L., COMTE RM. A fibrin selant for perforated and preperforated corneal ulcers. *Br. J. Ophthalmol.*, 1989, 73, 757-61.

25 LASA JR CI., KIDD RRD., NUNEZ HA., DROHAN WN. Effect of fibrin glue and opsite on open wounds in DB/DB mice. *J. Surg. Res.*, 1993, 54, 202-6.

- 26 LERNER R., BINUR NS. Current status of surgical adhesives. *J. Surg. Res.*, 1990, 48, 165-81.
- 27 LEUNG GY., PEONIS V., VARNELL ED., LAM DS., KAUFMAN HE. Preliminary *in vitro* evaluation of 2-octyl cyanoacrylate to seal corneal incisions. *Cornea*, 2005, 24, 998-9.
- 28 MESTER U., ZUCHE M., RAUBER M. Astigmatism after phacoemulsification with chamber lens implantation: small incision technique with fibrin glue adhesive for wound closure. *J. Cataract. Refractive. Surg.*, 1993, 19, 616-9.
- 29 MORANDINI W., ORTIZ V. Adesivos biológicos em cirurgia. *Acta Cir. Bras.*, 1992, 7, 80-5.
- 30 OKAMOTO T., ALVES-REZENDE MCR., CLÁUDIO CC., RODRIGUES TS., OKAMOTO R. Effects of tissucol and epsilon aminocaproic acid in the healing process following dental extraction in dehydrated rats. *Braz. Oral Res.*, 2006, 20, 1-10.
- 31 SAMPAIO RL., RANZANI JJT. Aplicação do adesivo sintético embucrilato (Hystoacril) na reparação de úlceras profundas da córnea. Estudo experimental em cães (*Canis familiares*, Linnaeus, 1758). *Braz. J. Vet. Res. Anim. Sci.*, 2005, 42, 438-49.
- 32 SCHOENAU JSF., PIPPI NL. Aspectos morfológicos e funcionais da córnea: uma breve revisão. *Hora Vet.*, 1993, 12, 49-53.
- 33 SIEDENTOP KH., PARK JJ., SANCHEZ B. An autologous fibrin tissue adhesive with greater bonding power. *Arch. Otolaryngol. Head Neck Surg.*, 1995, 121, 769-72.
- 34 SIERRA DH. Fibrin sealant adhesive systems: a review of their chemistry, material, properties and clinical applications. *J. Biomater. Appl.*, 1993, 7, 309-52.
- 35 SLATTER D. Cornea e esclera. In: SLATTER D. Ed. *Fundamentos de Oftalmologia Veterinária*. 3.ed. São Paulo: Editora Roca, 2005: 283-338.
- 36 STARTUP FG. Corneal ulceration in the dog. *J. Small Anim. Pract.*, 1984, 25, 737-52.
- 37 SZURMAN P., WARGA M., GRISANTI S., ROTERS S., ROHRBACH J., AISENBREY S., KACZMAREK RT., BARTZ-SCHIMIDT KU. Sutureless amniotic membrane fixation using fibrin glue for ocular surface reconstruction in a rabbit model. *Cornea*, 2006, 25, 460-6.

38 THOMAZINI-SANTOS IA., BARRAVIERA SRCS., MENDES-GIANNINI MJS., BARRAVIERA B. Surgical adhesives. *J. Venom. Anim. Toxins*, 2001, 7, 1-10.

39 WERNER LP., LEGEAIS JM. Les colles chirurgicales en ophtalmologie. *J. Fr. Ophthalmol.*, 1997, 20, 146-59.

40 WHEATON LG., GREENSHIELDS RM., MEYERS K., WARDROP KJ., MOORE M. Evaluation of canine-derived fibrin sealant as a hemostatic agent. *Vet. Surg.*, 1994, 23, 358-64.