Autologous Fibrin Membrane Combined With Solid Platelet-Rich Plasma in the Management of Perforated Corneal Ulcers

A Pilot Study

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Importance: The combined use of autologous fibrin membrane and the eye platelet-rich plasma (E-PRP) clot could be considered as a new surgical alternative for the closure of corneal perforations.

Objective: To evaluate the use of autologous solid platelet-rich plasma in combination with an autologous fibrin membrane as a surgical alternative for wound closure in perforated corneal ulcers.

Design: Both the fibrin membrane and the E-PRP clot were prepared with the patient’s own blood just before the operation. Nylon stitches were used to fixate the fibrin membrane to the conjunctiva and then the E-PRP clot was placed over the corneal perforation, underneath the fibrin membrane. A temporal partial tarsorrhaphy was performed at the end of the procedure. We conducted postoperative monitoring for 3 months.

Setting: Vissum Corporacion Oftalmologica, Alicante, Spain.

Participants: Eleven patients with perforated corneal ulcers.

Intervention: Surgical alternative for the closure of corneal perforation.

Main Outcomes and Measures: Corneal biomicroscopy, fluorescein test, digital tonometry.

Results: In all cases the corneal perforation was sealed. The fibrin membrane was present over the corneal surface for the first 3 to 5 days and then gradually disappeared. No evidence of infection or inflammation was detected. Digital tonometry confirmed acceptable levels of ocular tonus in all cases from day 2 after the operation. No patients reported pain, discomfort, or other symptoms, and no complications were observed. After 3 months’ follow-up, there was no evidence of relapses or perforations. Corneal grafting was eventually performed in 7 of the 11 cases.

Conclusions and Relevance: The combined use of autologous fibrin membrane and E-PRP clot is a safe and effective surgical alternative for the closure of corneal perforations. This technique can be considered as a temporary measure until the condition of the cornea permits definite intervention.


Corneal perforations can be the result of trauma or ulcerative disease of different sources. This condition is identified by the presence of a filtrating spot observed with the slitlamp following fluorescein staining of the ocular surface (Seidel phenomenon). The perforation can be spontaneous or appear when pressure is applied to the eye. In severe cases, corneal perforation is obvious; eye hypotony is observed and can eventually be partially sealed by the adherence of the iris and intraocular structures. All cases of corneal perforation constitute a major ophthalmic emergency necessitating the intervention of corneal surgeons because of the severity and major consequences, such as infection, severe anatomic distortion of the anterior segment of the eye, retinal detachment, phthisis bulbi, and total blindness.1

Corneal perforations have been managed with the use of sealants such as plastic ocular adhesives, including cyanoacrylate2,3 or tectonic drape patches.4 Conjunctival flaps also have been used to seal corneal perforations.5 However, this is a traumatic technique for the conjunctiva because it draws new vessels to the cornea and mutilates conjunctival tissue, which, in many cases, is necessary for natural main-
onformance of the ocular surface in chronically affected patients. Corneal grafting is usually delayed as much as possible because it is performed in inadequate clinical conditions, and in many cases it may not be possible because of the lack of adequate corneal tissue (ie, fresh corneal tissue or glycerol-preserved corneas). For all of these reasons, a practical, accessible, and low biological risk technique is needed to treat corneal perforation.

Amniotic membrane (AM) has been used successfully as an alternative for urgent management of corneal perforations before major corneal surgery and can be considered now as one of the preferred options. However, AM transplant has distinctive disadvantages. First, it is an uneven biological tissue, with many variables involved in its quality and biological capabilities. Second, it harbors major biological hazards because of the possible presence of viral contaminants and prions. Third, it is not universally available and, in many cases, is expensive. In addition, in some countries, AM requires specific authorization to be used as a grafting tissue. Because of these limitations, autologous tissue would be highly desirable, if possible, for use in the treatment of corneal perforations.

Previous studies have reported on the use of AM in combination with an autologous eye platelet-rich plasma (E-PRP) solid clot for surgical management of corneal perforations. Vissum Corporation created and registered the term E-PRP to describe a platelet-rich plasma preparation designed to treat ocular surface diseases and to differentiate it from other platelet-rich plasma preparations used in medicine. In the present report, we describe the combined use of an E-PRP solid clot with an autologous fibrin membrane for sealing corneal perforations related to different severe corneal ulcerative diseases. To the best of our knowledge, this is the first report of an autologous fibrin membrane being used successfully in these difficult, urgent cases.

METODS

Eleven cases with different types and degrees of corneal perforations were treated using a combination of autologous fibrin membrane and platelet-rich plasma clot. All patients, except one, had experienced a central corneal perforation caused by a chronic corneal ulcerative disorder. Only 2 patients had a history of corneal superficial infection before the perforation. In all cases, the cornea was identified as perforated by spontaneous Seidel test using fluorescein tear staining. The size of the perforations ranged from 1.0 to 2.0 mm. In 3 cases, the perforation was partially blocked by intraocular tissue, particularly the iris. The anterior chamber was present in all but one patient who had a cataract intraocular lens impacted in the perforation at the time of the operation. All patients received prophylactic treatment with topical antibiotics (second-generation fluoroquinolones) when the perforation was diagnosed. None of them demonstrated signs of active infection at the time of the operation. The Table reports the diagnosis of these patients and the most relevant clinical data associated with the corneal perforation.

PREPARATION OF THE SOLID E-PRP CLOT

For preparation of the E-PRP clot, 40 to 60 mL of blood was obtained from the patient just before the operation. The blood was collected in 10-mL sterile tubes containing 1 mL of sodium citrate, 3.2%, to avoid coagulation. The tubes were then centrifuged in a 1-step process at 5°C, 1600 rpm for 10 minutes. In these conditions, 2-part plasma is obtained: the upper part consists of platelet-poor plasma and the lower part consists of platelet-rich plasma (PRP). The platelet-poor plasma is aspirated and reserved to prepare the fibrin membrane, and the plasma nearest to the red cells (PRP) is harvested to prepare the E-PRP clot. At that time, 1 mL of the PRP is placed into each well of a 4-well tissue culture plate (Nunc; Thermo Scientific), and 50 μL of calcium chloride, 10% (Braun), is added to each well for activation. After the plasma is mixed carefully with a sterile pipette, the plates are incubated at 37°C for 30 minutes. At that time, the E-PRP clot is formed and ready to be applied immediately onto the ocular defect. In the E-PRP clot, the ratio of platelet enrichment is about 2 to 3 times more than the full blood values. Manipulation of the tubes to obtain the E-PRP must be performed under strict sterile conditions using a laminar flow hood.

PREPARATION OF THE AUTOLOGOUS FIBRIN MEMBRANE

For preparation of the autologous fibrin membrane, we used an appropriate glass beaker previously sterilized. Working inside the laminar flow hood, we placed 5 mL of platelet-poor plasma in the beaker; 500 μL of calcium chloride, 10%; and 1 mL of previously prepared autologous thrombin. The autologous thrombin was prepared by activating 3 mL of E-PRP with 300 μL of calcium chloride, 10%, and incubating it at 37°C for 30 minutes. After the contents were mixed carefully, the beaker was incubated at 37°C for 1 hour. During that time, the plasma fibrinogen, which is soluble, was converted by thrombin into fibrin, which is insoluble and viscous. After the incubation period, the fibrin membrane obtained was circular, with a diameter between 18 and 22 mm, and its thickness was approximately 1 mm. With this shape and size, the fibrin membrane was perfectly manageable and suitable for applying to the damaged ocular surface (Figure 1).

SURGICAL TECHNIQUE

In all cases, the operation was performed under retrobulbar anesthesia (mepivacaine hydrochloride, 2%; B. Braun Melsungen AG), using a maximum of 4 mL with an Atkinison intraconal retrobulbar needle (Sharpoint 38 mm-25 G; Equipsa). The eyes were rinsed with povidone-iodine aqueous solution, 20%, 30 minutes after the regional anesthesia was administered. The eyelids were opened with an eyelid speculum (Vissum eyelid speculum; Epsilon), and the epithelium was debrided with a sponge 1.0 to 1.5 mm from the perforation. The fibrin membrane was then dried on an absorbent sterile paper until it appeared to be a solid, fibrous structure. A 10-0 nylon running suture was used to sew the fibrin membrane to the 180° inferior conjunctiva. In 3 cases, interrupted sutures were used for the purpose (monofilament polyamide 10-0; Arag) (Figure 2). Solid platelet-rich plasma clots (E-PRP solid) were then placed onto the corneal perforation and the epithelial debrided area underneath the fibrin membrane. Additional stitches were used to fixate the fibrin membrane to the remainder of the conjunctiva. The fibrin and E-PRP clots were placed in the same manner when there was iris or other intraocular tissue blocking the perforation. In cases in which an intraocular lens was blocking the perforation, the patch procedure was the same, the intraocular lens was repositioned, and the anterior chamber was filled with viscoelastic. At the end of the procedure, a temporal partial tarsorrhaphy was performed to allow observation of the perforation.
the central cornea with the slitlamp. The steps of the procedure are shown in Figure 2.

Tobramycin topical ointment (Tobrex; Alcon Cusi) was applied to the eyelid borders during the postoperative period. The patients received systemic antibiotics (ciprofloxacin hydrochloride, 750 mg every 12 hours for 5 days) and, if not contraindicated, a nonsteroidal anti-inflammatory drug (800 mg of ibuprofen every 8 hours for 3 days). The borders of the eyelid were rinsed with normal saline every 8 hours, followed by topical application of the tobramycin ophthalmic ointment.

POSTOPERATIVE FOLLOW-UP

All patients were observed daily for 10 days. Particular care was taken to note the presence of the fibrin membrane between the opening of the eyelids allowed by the tarsorrhaphy. Any evidence of infection or signs of inflammation was evaluated. The ocular tonus was assessed by pressing the eye gently with the fingers (digital tonometry).

RESULTS

All 11 patients had developed corneal perforation secondary to a severe corneal ulcerative disease. In all cases, the corneal perforation was sealed. The fibrin membrane was present on the corneal surface for the first 3 to 5 days and then gradually disappeared. No evidence of infection or inflammation was detected in any of the cases. Finger pressure confirmed the presence of acceptable levels of ocular tonus in all cases from day 2 after the operation. The patients did not report pain, discomfort, or any other symptoms during the postoperative period.

After 7 days, the temporal tarsorrhaphy was removed from all patients and the ocular surface was ex-

Table. Characterization of 11 Patients With Corneal Ulcer Perforation Treated With the Combined Use of Eye Platelet-Rich Plasma Clot and Autologous Fibrin Membrane

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Diagnosis</th>
<th>Cause of Perforation</th>
<th>Associated Findings</th>
<th>Initial VA</th>
<th>Outcomes at 7 d</th>
<th>Final VA</th>
<th>Penetrating Keratoplasty</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/66</td>
<td>Perforation in corneal graft</td>
<td>Trauma caused by a vegetable, endophthalmitis, desemetocoele</td>
<td>Moderate inflammation, corneal melting</td>
<td>LP</td>
<td>Epithelial closure, stable stromal thinning, inflammation decreased</td>
<td>LP</td>
<td>Yes</td>
</tr>
<tr>
<td>2/F/72</td>
<td>Perforation in corneal graft</td>
<td>Corneal ulcer, graft thinning</td>
<td>Severe dry eye, corneal melting</td>
<td>LP</td>
<td>Epithelial closure, stable stromal thinning</td>
<td>HM</td>
<td>Yes</td>
</tr>
<tr>
<td>3/F/50</td>
<td>Perforation in lamellar corneal graft</td>
<td>Neurotrophic ulcer, trigeminal ablation</td>
<td>Dry eye, moderate inflammation, corneal melting</td>
<td>0.3</td>
<td>Total corneal wound healed, inflammation decreased</td>
<td>0.1</td>
<td>No</td>
</tr>
<tr>
<td>4/M/56</td>
<td>Perforation in corneal graft</td>
<td>Ocular cicatricial pemphigoid</td>
<td>Conjunctivalization, vascularization, corneal melting</td>
<td>HM</td>
<td>Epithelial and stromal closure</td>
<td>LP</td>
<td>Yes</td>
</tr>
<tr>
<td>5/M/23</td>
<td>Perforation in corneal graft</td>
<td>Sicca keratoconjunctivitis</td>
<td>Conjunctival hyperemia, dry eye, vascularization</td>
<td>NM</td>
<td>Total corneal wound healed, inflammation decreased</td>
<td>HM</td>
<td>No</td>
</tr>
<tr>
<td>6/F/47</td>
<td>Perforation in corneal graft</td>
<td>Persistent epithelial defect</td>
<td>Calcific keratopathy</td>
<td>LP</td>
<td>Epithelial closure, stable stromal thinning</td>
<td>LP</td>
<td>Yes</td>
</tr>
<tr>
<td>7/F/82</td>
<td>Perforation in corneal graft</td>
<td>Fuch dystrophy</td>
<td>Corneal edema, epithelial bulla, persistent epithelial defect</td>
<td>HM</td>
<td>Epithelial closure, stable stromal thinning</td>
<td>HM</td>
<td>Yes</td>
</tr>
<tr>
<td>8/M/71</td>
<td>Perforation in corneal graft</td>
<td>Chronic paracentral corneal melting</td>
<td>Inflammation, severe pain</td>
<td>0.1</td>
<td>Epithelial and stromal closure, inflammation decreased</td>
<td>0.3</td>
<td>Yes</td>
</tr>
<tr>
<td>9/F/56</td>
<td>Perforation in corneal graft</td>
<td>Neurotrophic ulcer, trigeminal ablation</td>
<td>Dry eye, large central ulcer</td>
<td>HM</td>
<td>Epithelial closure, inflammation decreased</td>
<td>HM</td>
<td>No</td>
</tr>
<tr>
<td>10/M/72</td>
<td>Perforation in corneal graft</td>
<td>Rheumatic</td>
<td>Dry eye, central ulcer</td>
<td>HM</td>
<td>Epithelial and stromal closure</td>
<td>0.4</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: CF, counting fingers; HM, hand motion; LP, light perception; NM, not measurable; VA, visual acuity.
tensively inspected. In 3 cases, partial remains of the fibrin membrane were observed at the bottom of the previously existing perforation (Figure 3). In all cases, the corneal perforation was sealed, with no evidence of leakage even when moderate pressure was applied to the globe (negative spontaneous or forced Seidel test) (Figure 4 and Figure 5). All eyeballs had good tonus by finger control inspection and had no signs of active inflammation (neither ciliary injection nor conjunctival edema was observed). The nylon stitches were removed with forceps and microscissors. Only 1 eye had synchiae at the end of the follow-up period.

The Table reports a different degree of corneal wound healing at 7 days postoperatively. In some cases, we observed epithelial closure over a stable but marked stromal thinning (patients 1, 2, 7, 8, and 10); others showed healing of both the epithelium and stroma (patients 3, 4, 5, 6, 9, and 11). The minimum follow-up period after the operation was 3 months, and all patients who underwent penetrating keratoplasty received corneal grafting after that period. The Table lists visual acuities documented before treatment of the perforation and those determined at the end of the third postoperative month. No relapses of the ulcerative corneal condition or perforation occurred in any of the cases before the definite corneal grafting surgery that was performed in 7 of the
11 patients. Unfortunately, we did not receive the pathologic examination results because the patients returned to their local hospital to undergo keratoplasty.

COMMENT

Platelets play a central role in hemostasis, promoting coagulation in vascular injuries. At the same time, platelets constitute natural reservoirs of growth factors, cell adhesion molecules, and cytokines stored in their alpha granules. Between these growth and mitogenic factors are epidermal growth factors, platelet-derived growth factors, fibroblast growth factors, insulinlike growth factors, and transforming growth factors. They also contain important cell adhesion molecules, such as fibrin, fibronectin, and vitronectin, which enhance growth factor activities. The release of these molecules at the site of injury helps initiate and modulate wound healing, thus promoting tissue reparation in both soft and hard tissues.

Autologous platelet-rich preparations have been used successfully in medicine since 1999 as a component for tissue regeneration procedures, such as oral and maxillofacial surgery, tendon repair, articular surgery, and reversal of skin ulcers. In dental surgery, some authors have described the use of an autologous platelet-rich clot sealed with a fibrin membrane after tooth extraction, demonstrating acceleration in ossification, marked reduction in the time required for implant stabilization, and improvement in success rates. In the same study, the presence of high amounts of several growth factors released from platelets was reported, and its synergic action in bone regeneration was suggested.

The presence of corneal receptors for numerous growth factors contained in platelet granules has been demonstrated. The effect of these growth factors in promoting migration, mitosis, and differentiation of corneal cells as well as extracellular matrix production also has been shown.

Based on this, we described E-PRP as an autologous platelet-rich formulation designed to treat ocular surface disorders. We found that E-PRP prepared using our methods yields a 1.6- to 2.5-fold enrichment of platelets when compared with full blood. We assume that this formulation contains the proper growth factors of blood and platelets, although their concentration was not measured in this study. In a prospective observational study in patients with severe dry eye, Alio et al showed that...
89% of patients reported a significant improvement in symptoms using the E-PRP. There was also improvement in the quality of the tear film and conjunctival hyperemia, with significant improvement in corneal fluorescein staining. In another prospective study, Alio et al\textsuperscript{11} used E-PRP to treat severe symptomatic ocular surface syndrome after laser-assisted in situ keratomileusis. They reported good or excellent improvement in the symptoms in 84% of the patients, with benefits in the quality of the tear film and in best-corrected visual acuity. Sixty-nine percent of the patients showed complete resolution of punctate keratitis. Alio et al\textsuperscript{11} have also reported on the use of E-PRP in the treatment of dormant corneal ulcers, reporting significant clinical improvement in 92% of the eyes, with complete healing of the ulcer in 50% of the cases. In the same study, E-PRP as a clot was used as a surgical tool for eyes with perforated corneas or with a high risk of perforation. The clot was placed under a patch of amniotic membrane to enhance the growth factor action at the site of injury. Seventy-one percent of these cases healed completely.

Multilayer AM transplant has been one of the surgical techniques proposed to seal corneal perforations.\textsuperscript{8} However, the exact mechanism of action is not known, and variable outcomes are obtained in the treatment of different ocular surface diseases, including corneal perforations. In treatment of neurotrophic ulcers, Khokhar et al\textsuperscript{12} found no significant difference in epithelization and subsequent healing using AM or conventional therapies such as bandage contact lens or tarsorrhaphy. It has been demonstrated\textsuperscript{13} that the method of processing and preserving the membrane can affect its biological properties and possibly its efficacy. Kruse et al\textsuperscript{14} suggested that the preservation of AM in glycerol kills epithelial cells, and it is unclear whether the stromal cells and growth factors survive cryopreservation. These aspects as well as interdonor variations could explain variable outcomes and should be considered in an attempt to standardize AM handling procedures. Also, AM contains both anti-inflammatory and proinflammatory cytokines and other molecules with contradictory actions. For instance, it contains interleukin 10 (IL-10) and IL-1x, which have anti-inflammatory actions, and IL-6 and IL-8, which promote an inflammatory status.\textsuperscript{15} Finally, AM represents a heterologous transplant that requires strict serologic analysis to avoid the transmission of infectious diseases.

In this study, we substituted the AM used in a previous report\textsuperscript{11} for an autologous fibrin patch to maintain the E-PRP clot attached to the margins of perforated corneas. If we compare this new technique (E-PRP and autologous fibrin clot) with E-PRP clot and AM, the main difference is the easy handling of fibrin membrane because of its consistency and thickness. However, the main advantage is that it is 100% autologous. The fibrin strands of the patch can bind to the clot and at the same time to the stromal collagen fibers of the cornea, thus contributing to sealing the defect. The fibrin patch and platelet clot gradually disappear over the wound, constituting a physiologic and biologically active solution for corneal perforation.

Partial tarsorrhaphy was chosen to maintain the fibrin membrane and clot attached at the ocular surface to avoid eyelid movements, thus facilitating wound healing. At the same time, the patient’s discomfort diminished, allowing us to open the eyelids wide enough to detect any signs of infection or inflammation. We observed closure of corneal perforations in all cases with different degrees of corneal wound healing and improved anatomic outcomes in the less severe cases.

The main disadvantage of this technique would be the cost, especially when compared with the cost of cyanoacrylate glue. Specialized technicians are needed to prepare the autologous fibrin membrane and E-PRP clot, and the process requires at least 2 hours of laboratory work.

Our findings suggest that the combined use of autologous fibrin membrane and E-PRP clot is a safe and effective alternative for the closure of corneal perforations. The obvious advantage of this technique is the use of autologous material for surgery. Considering that the preparation of E-PRP and fibrin membrane is possible in most hospitals, its apparent efficacy and autologous nature certainly deserve further studies to confirm its role for emergency management of corneal perforations related to chronic ulcerative corneal disorders.

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