

# Comparative Study Between Fibrin Glue Prepared from a Single Human Donor and Sutures for Sealing Induced Corneal Penetrating Wounds on Porcine Eyes

Anne Marie Macasaet, MD<sup>1</sup>, Raymond Nelson Regalado, MD<sup>1</sup>, Irwin Cua, MD<sup>1</sup>

<sup>1</sup>St. Luke's Medical Center – Quezon City  
Quezon City, Philippines

Correspondence: Anne Marie Macasaet, MD  
St. Luke's Medical Center, 279 E. Rodriguez Sr. Blvd.  
Quezon City, 1102, Philippines  
Email: annemariemacasaet@gmail.com

Disclaimer: Authors have no financial interest in this study.

## ABSTRACT

**Objective:** To determine the efficacy of fibrin glue derived from a single human donor for sealing induced penetrating corneal wounds on cadaveric porcine eyes compared to conventional suturing.

**Methods:** Forty (40) porcine eyes were randomized into two groups. In the experimental group, the corneal incisions were sealed using fibrin glue prepared from a single human donor. Eyes in the control group were sealed using two interrupted nylon 10-0 sutures. Baseline intraocular pressures were obtained and an anterior chamber maintainer connected to the Centurion Vision System was inserted through a side port. A 3 mm metal keratome was used to create a uniform, central, full-thickness straight incision in all eyes. After making the incision, the presence or absence of leakage was determined using Seidel's test. The eyes were then sealed according to the group they were randomized in. The intraocular pressure (IOP) was gradually increased using the Centurion Vision System to determine the leakage pressure of all sealed eyes in both groups.

**Results:** The mean baseline IOP was comparable between the two groups (control group =  $21.15 \pm 1.66$  mmHg; experimental group =  $21.65 \pm 1.81$ ,  $p$  value = 0.299). All eyes in the control group, showed no leakage immediately after sealing the corneal wounds with sutures. One out of twenty eyes in the experimental group was positive for leakage after application of the fibrin glue ( $p$  value = 0.5). The mean leakage pressure was significantly higher for the suture group at  $90.25 \pm 14.9$  mmHg compared to the fibrin glue group at  $32.30 \pm 7.6$  mmHg ( $p$  value =  $<0.001$ ).

**Conclusion:** Fibrin glue prepared from a single donor is comparable to two nylon 10-0 sutures in sealing 3 mm corneal penetrating wounds at IOP of up to 32 mmHg. For higher IOP, nylon 10-0 suture is more effective than fibrin glue in sealing the corneal penetrating wounds in cadaveric porcine eyes.

**Keywords:** Corneal lacerations, corneal penetrating injury, corneal perforations, autologous fibrin glue, fibrin glue, tissue adhesives

Biologic adhesives, namely fibrin glue, have been used for various indications in the fields of dermatology, surgery, urology, neurosurgery and ophthalmology. It is mainly used for hemostasis and as a sealant for breaks and anastomoses. It has since been proven to be comparable with sutures, which have long been the gold standard, but with the added benefit of shorter operating time and ease of use<sup>1</sup>.

Various studies have been done comparing fibrin adhesives to conventional sutures for numerous clinical indications. Some of these have investigated the efficacy of tissue adhesives for sealing corneal wounds and perforations. A study by Bannitt *et al*, compared commercially prepared fibrin glue and cyanoacrylate tissue adhesive with sutures and found that both fibrin glue and cyanoacrylate can be a stable alternative to conventional sutures in the closure of clear corneal incisions<sup>2</sup>. A randomized controlled trial by Sharma *et al*, concluded that commercially prepared fibrin glue and cyanoacrylate tissue adhesive were found to be both effective in the closure of corneal perforations up to 3 mm in diameter, with fibrin glue providing faster healing and inducing significantly less corneal vascularization<sup>3</sup>. Both of these studies used commercially prepared fibrin glue which is manufactured from pooled human plasma with the addition of an anti-fibrinolytic agent coming from a bovine source. Although relatively safe, some patients develop adverse reactions to commercially prepared fibrin glue<sup>4,6</sup> and reports have been made on the possible risk of transmission of blood-borne viral diseases from its use<sup>7</sup>. Hence, further studies have investigated on the development of fibrin glue from an autologous source to minimize the risk of infection and immunologic reactions<sup>8-10</sup>.

In ophthalmology, the use of autologous fibrin glue has been studied for conjunctival autografts after pterygium excision and showed favorable results<sup>11</sup>. However, at present, no studies have been made regarding the use of autologous fibrin glue for corneal lacerations.

Autologous fibrin glue presents the main advantage of minimizing the risk for blood-borne disease transmission and reduces the possibility of an immune reaction to the product. Although sutures are still the gold standard for sealing corneal perforations, fibrin glue provides several advantages over sutures. Fibrin glue eliminates the need for suture removal, may cause less induced post-operative corneal astigmatism and less scar formation from suture tracts.

It is also a cheaper alternative to the more expensive commercially prepared fibrin adhesives.

Our main objective is to determine the efficacy of fibrin glue derived from a single human donor for sealing induced corneal penetrating wounds on porcine cadaver eyes compared to conventional suturing. The presence or absence of leakage in eyes sealed with fibrin glue will be compared to eyes sealed with sutures. Leakage pressures defined as the intraocular pressure (IOP) at which a positive Seidel's test is noted after increasing the IOP, will also be determined and compared between the two groups.

## METHODOLOGY

This is an in vitro comparative experimental study using porcine eyes. All eyes were procured within 12 hours post-mortem. Eyes with corneal pathologies, such as corneal opacities or evidence of previous trauma, were excluded. The porcine eyes were randomized in a 1:1 ratio to either fibrin glue or sutures.

The components of the fibrin glue, i.e., fibrinogen concentrate and thrombin, were collected from a single human donor and processed in the laboratory. The voluntary donor had no known co-morbidities and no history of bleeding disorders or coagulation factor deficiencies.

The method for processing of autologous fibrin glue was adopted from the technique proposed by Hirsch *et al*<sup>12</sup>. Whole blood was obtained by phlebotomy and was collected in a single blood bag with CPDA-1 as anticoagulant (Teruflex, Terumo Medical Corp., USA). The whole blood was then transferred into eight conical centrifuge tubes. Cellular components were removed by centrifugation at 2000G for 15 minutes to produce plasma. The plasma was then aspirated and transferred into another set of conical tubes for further processing. Fibrinogen was separated from the plasma by cryoprecipitation to produce a fibrinogen-containing precipitate and a thrombin-containing supernatant. The plasma was frozen at a temperature of -70°C. After freezing, the plasma was thawed at 4°C and subsequently centrifuged at 5000G for 10 minutes. The supernatant was then aspirated and transferred into another set of conical tubes. The remaining precipitate served as the fibrinogen component of the fibrin glue and was then collected in a 1 cc syringe for subsequent application to the area of the corneal incision.

The residual fibrinogen in the supernatant was removed by clotting to produce serum containing thrombin and fibrin. Clotting of the residual fibrinogen was accomplished by adding 0.1 volume of 0.2M calcium chloride (Fame Analytical Reagents and Laboratories, Philippines). The thrombin containing serum was then aspirated from the clotted fibrin into a 1 cc syringe and was used as the thrombin component of the fibrin glue.

At the start of the experiment, the porcine eyes were placed in a globe holder. Baseline IOP were obtained using a Tonopen (Reichert Technologies, USA) and results were recorded. A side port incision was created along the peripheral cornea using a standard 15 degree metal keratome (SatinSlit, Alcon Laboratories, USA). A gauge 21 anterior chamber maintainer connected to the Centurion Vision System (Alcon Laboratories, USA) was inserted through the side port with the infusion turned off (*Figure 1*). A straight incision perpendicular to the central corneal surface was made using a standard 3 mm metal keratome (Laseredge, Storz, USA) (*Figure 2*). Seidel's test was done to assess for wound leakage at the 3 mm incision site using a sterile fluorescein strip (BioGlo, HUB Pharmaceuticals, USA). Egress of fluid was recorded as a positive Seidel's test. (*Figure 3*)



Figure 1. AC maintainer attached to Centurion Vision System



Figure 2. Creation of 3mm central corneal incision



Figure 3. Positive Seidel's test after creation of incision

In the study group, the corneal incision was sealed using the previously prepared fibrin glue. The fibrinogen and thrombin components of the fibrin glue were placed in separate 1 cc syringes with gauge 27 cannulas. The incision site was dried by gentle wiping with a cellulose sponge prior to application of the fibrin glue. Equal amounts, approximately 0.1 mL each, of the fibrinogen and thrombin solutions were placed simultaneously over and within the incision site. The fibrin glue was then allowed to set for 3 minutes. Seidel's test was done to check for leaks after application of fibrin glue. If no leakage was noted, the intraocular pressure was then manipulated using the Centurion Vision System. The intraocular pressure was set at 34 mmHg initially. If no leak was noted, the IOP was then increased by increments of 5, and the IOP at which leakage occurs was determined and labeled as the leakage pressure.

Documentation of wound leakage after sealing the incision and manipulation of intraocular pressure was done. Any sign of leakage through the incision was recorded as positive. (*Figures 4 and 5*)

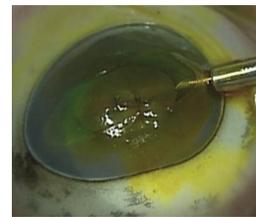


Figure 4. Negative Seidel's test after sealing with sutures

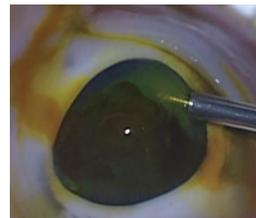


Figure 5. Negative Seidel's test after sealing with fibrin glue

In the control group, the corneal incisions were closed using two partial thickness simple interrupted sutures with Nylon 10-0 suture (Ethilon, Alcon Laboratories, USA). Seidel's testing and manipulation of IOP were done as previously described.

The results were tabulated using Microsoft Excel and data were statistically analyzed. The mean baseline intraocular pressures, as well as the leakage pressures between the two groups were analyzed using the

Mann-Whitney Test for two independent variables where a p-value of less than 0.05 was considered significant (95% CI). Data for the presence of leakage after sealing was analyzed using Fisher's Exact test and a p-value of less than 0.05 was considered significant (95% CI).

## RESULTS

The baseline intraocular pressures of the suture group were compared to the fibrin glue group. The mean baseline IOP was  $21.15 \pm 1.66$  mmHg for the suture group and  $21.65 \pm 1.81$  mmHg for the fibrin glue group (p value = 0.299).

All eyes in the control group, showed no leakage immediately after sealing the corneal wounds with sutures. One out of twenty eyes in the experimental group was positive for leakage after application of the fibrin glue (p value = 0.5).

**Table 1. Seidel's test results after wound sealing in both groups**

Seidel's Test after wound sealing			
	Positive	Negative	Total
Suture Group	0	20	20
Fibrin Glue Group	1	19	20
Total	1	39	40

P value = 0.5

The mean leakage pressure for the suture group was  $90.25 \pm 14.9$  mmHg and the mean leakage pressure for the fibrin glue group was  $32.30 \pm 7.6$  mmHg. (p value = <0.001).

## DISCUSSION

The baseline IOP measurements were comparable between the two groups. All eyes in the control group were negative for leakage after sealing the corneal wounds with sutures while one out of twenty eyes in the experimental group was positive for leakage after application of the fibrin glue. This may be due to inadequate drying of the wound area prior to application of the glue or it could be due to insufficient deepithelialization of the area where the glue was to be applied. Statistical analysis showed no association between the presence of leakage and method of wound sealing, indicating that sutures and fibrin glue have the same efficacy in sealing

wounds at intraocular pressures below baseline values.

The IOP was initially set at 34 mmHg using the Centurion Vision System, since this was reported to be the mean IOP under normal physiologic stresses such as coughing, straining or Valsalva maneuver<sup>13</sup>. The mean leakage pressure of the suture group was significantly higher compared to the fibrin glue group of which all sealed eyes in the experimental group showed leakage at 34 mmHg. This finding may indicate that the tensile strength of the fibrin glue prepared through the method described may not be strong enough to withstand pressures above 34 mmHg. Several other methods for preparation of autologous fibrin glue have been described in previous studies. In a study by Cavichiolo *et al*, they compared the tensile strength and local toxicity of fibrin glue prepared by three different techniques. They concluded that all methods produced good results as far as clot formation and non-toxicity, but ammonium sulfate precipitation produced the best tensile strength among the described methods of preparation, followed by cryoprecipitate and lastly by plasma fibrinogen<sup>14</sup>. Although evidence from this study suggests that ammonium sulfate precipitation produces the highest tensile strength among the three, the method for preparation of this adhesive may not be readily available in a conventional blood bank. Cryoprecipitate, on the other hand, is readily processed in the blood bank and provides a sufficient amount of fibrinogen for use as a component of fibrin glue, hence the reason why we chose this method of fibrin glue preparation for this study.

We can only infer that the results may be comparable when used in human eyes since porcine eyes were used in this experimental animal study. The determination of the leakage pressures were initially set at the mean IOP under normal physiologic stresses. The authors recommend testing the strength of the glue under normal intraocular pressures. Also, the method of preparation of the fibrin glue used in the study was only one of the techniques available for the production of fibrin glue. Various other methods for the preparation of autologous fibrin glue are available and may be worth investigating.

## CONCLUSION

Fibrin glue prepared from a single donor is comparable to two nylon 10-0 sutures in sealing

3 mm corneal penetrating wounds at IOP of up to 32 mmHg. For higher IOP, nylon 10-0 suture is more effective than fibrin glue in sealing the corneal penetrating wounds in cadaveric porcine eyes.

## REFERENCES

1. Vyas S, Kamdar S, Vyas P. Tissue adhesives in ophthalmology. *J Clin Ophthalmol Res* 2013;1:107-12.
2. Banitt M, Malta JB, Soong HK, et al. Wound integrity of clear corneal incisions closed with fibrin and N-butyl-2-cyanoacrylate adhesives. *Curr Eye Res* 2009;34:706-10.
3. Sharma A, Kaur R, Kumar S, et al. Fibrin glue versus N-butyl-2-cyanoacrylate in corneal perforations. *Ophthalmology* 2003;110:291-8.
4. Kober BJ, Scheule AM, Voth V, et al. Anaphylactic reaction after systemic application of aprotinin triggered by aprotinin-containing fibrin sealant. *Anesth Analg*. 2008;107:406-9.
5. Shirai T, Shimota H, Chida K, et al. Anaphylaxis to Aprotinin in Fibrin Sealant. *Intern Med*. 2005;44:1088-9.
6. Scheule AM, Beierlein W, Wendel HP, et al. Aprotinin in fibrin tissue adhesives induces specific antibody response and increases antibody response of high-dose intravenous application. *J Thorac Cardiovasc Surg*. 1999;118:348-53.
7. Hino M, Yamamura R, Nishiki S, et al. Human parvovirus B19-induced aplastic crisis in a patient treated with fibrin sealant. *Rinsho Ketsueki*. 1999;40:145-9.
8. Rock G, Neurath D, Semple E, et al. Preparation and characterization of human thrombin for use in a fibrin glue. *Transfusion Med* 2007;17:187-191.
9. Rostron CK. Epikeratophakia grafts glued with autologous cryoprecipitate. *Eur J Implant Refract Surg*. 1989;1:105-8.
10. Quigley RL, Perkins JA, Gottner RJ, et al. Intraoperative procurement of autologous fibrin glue. *Ann Thorac Surg*. 1993;56:387-9.
11. Anbari AA. Autologous cryoprecipitate for attaching conjunctival autografts after pterygium excision. *Middle East Afr J Ophthalmol*. 2013;20:239-243.
12. Hirsh J, Johnston M, Teoh K. 1996. Autologous fibrin glue and methods for its preparation and use. *US patent*. WO 1996031245 A1.
13. Bain WE, Maurice DM. Physiological variations in the intraocular pressure. *Trans Ophthalmol Soc UK*. 1959;79:249-260.
14. Cavichiolo, JB, Buschle M, Carvalho B. Comparison of fibrin adhesives prepared by 3 different methods. *Int Arch Otorhinolaryngol*. 2013;17:62-65.