Original article

Introduction of a potent single-donor fibrin glue for vascular anastomosis: An animal study

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Background: Vascular anastomosis is considered as a difficult surgical procedure. Although different alternative methods have been tried to tackle these difficulties, none were found to be successful. Commercial fibrin glue has recently been used for vascular anastomosis. However, it did not gain popularity due to some limitations such as low tensile strength, rapid removal by the immune system, and risk of transmission of blood-borne viral infections. In this article, we presented a novel method for producing single-donor human fibrin glue and determined its success rate for vascular anastomosis in an animal model. **Materials ans Methods**: In this study, 3 mL of single-donor fibrin sealant was prepared from 150 mL of whole blood containing 50-70 mg/mL of fibrinogen. The study was performed on 10 dogs and 5 cats. After transection of the carotid artery, both ends were anastomosed by means of 3-4 sutures (Prolene 8-0). The suture line was then sealed with one layer of the new fibrin sealant. After 3-8 weeks, the site of anastomosis was evaluated angiographically and morphologically for healing and possible complications such as thrombosis or aneurysm. **Results:** In evaluations 3 weeks after the surgery, all arterial anastomoses were patent in dogs, but some degree of subintimal hyperplasia was noted. After 8 weeks, all anastomoses were patent and the degree of subintimal hyperplasia was decreased. In cats on the other hand, after 4 weeks, all anastomoses were patent and subintimal hyperplasia was absent. **Conclusions:** Single-donor fibrin glue was a quite reliable and practical alternative to minimize suturing and therefore operative time in our animal model. This sealant can easily be obtained from the patient's whole blood. Its application in humans would require further studies.

Key words: Single-Donor Fibrin Glue, Commercial Fibrin Sealant, Vascular Anastomosis.

INTRODUCTION

So far, interrupted suturing has been the most popular and acceptable method for vascular anastomosis. This technique however, does have some limitations and adverse effects of its own.[1] Acland categorized the limitations leading to unsuccessful results into 5 main categories including tearing of vascular edge, blood leakage from the suture line, causing blood clot formation with intravascular extension, stricture formation due to the pressure imposed by the suture on the two ends, posterior arterial wall interposition, and intraluminal adventitial interposition.[2]

All of the above side effects are technical in nature. Furthermore, blood leakage from the space between sutures is not an uncommon problem. Because of these limitations, different alternative methods such as using rings, clips, laser, and tissue sealant have been introduced. Rings or clips are good methods but they are also technique-related and need training and expertise. In addition, variations in vascular diameter necessitate devices of different sizes, which is not practical for all centers. Inversion of vascular edge is also a major challenge in these methods. In laser surgery, high cost and aneurysm formation are the main disadvantages.[3-5] In 1962, Nakayama used a metallic ring that could be fixed in place and was used as a permanent implant,[6] but this method was only effective in smooth vascular edge with approximately equal size at both ends.

Another option is to use tissue glues. Two main types of glues, including cyanoacrylate glues and fibrin-based glues, are currently being used in surgery. Cyanoacrylate causes media necrosis. Moreover, this product causes irregularities in vessel wall and induces severe inflammation.[7] This type of glue is only applicable in dry fields which is not consistent with most vascular surgery occasions. US Food and Drug Administration (FDA) approved this type of glue only for local and topical use and disapproved it as an internal tissue glue.[5]

Fibrin glue is a general term to denote all fibrin-based sealants. The first report of using this type of glue dates back to early 20th century.[8] In 1940, Young and Medawar used bovine thrombin and

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Received: 15-04-2012; **Revised:** 15-05-2012; **Accepted:** 27-05-2012

fibrinogen for nerve anastomosis[9] and Tidrick used this type of glue for skin graft immobilization.[10] However, none of these studies were successful.

Matras et al.[11] and Pearl et al.[12] reported the first clinical trials of fibrin sealant in microsurgery. Thereafter, many articles have been published about the effectiveness of this glue in vascular anastomosis to reduce the number of sutures and time of surgery, and to minimize trauma to vessel wall. However, the results have been controversial, from very successful[13-15] to very unsatisfactory having complications such as thrombosis and aneurysmal changes at the site of anastomosis.[16]

Single-donor fibrin glue is identical to other fibrin glues in nature and is derived from a single blood sample. Hence, there is no risk of hyper-sensitivity or infection transmission. The main challenge is to obtain an effective sealing producing efficient adhesion and tensile strength. The best protocol to produce the desired fibrin glue would be the one with a high fibrinogen yield. An appropriate method should have the following criteria:
1) It should be performed with a reasonable and minimal amount of whole blood;
2) The processing equipment should be easily available and not complicated;
3) A close system should be used for preparation to prevent contaminations.

In this article, a new preparing protocol for single-donor fibrin glue with high fibrinogen content and high growth factor is introduced. We determined the application of this glue for vascular anastomosis.

MATERIALS AND METHODS

This experimental study was performed on 10 dogs. The dogs were of Iranian race, weighed 15-20 kg, and aged 2-3 years old. The same anesthetic and surgical procedures were performed on all dogs. Arterial anastomosis was performed under general anesthesia with ketamine (10 mg/kg) and acepromazine as sedative (2 mg/kg). Anesthesia was continued with tracheal intubations and 1.5% halothane. With a longitudinal incision along the anterior border of the sternocleidomastoid muscle, the skin was incised and the carotid artery was exposed. The study protocol was approved by our local ethics committee.

Autologous fibrin was first prepared from the dogs' whole blood. A modified version of the method introduced by Thorn et al.[17] was employed to prepare fibrin glue from approximately 150 mL of whole blood. Fibrin sealant derived by the classic method of Thorn et al.[17] does not produce enough adhesiveness as it will be discussed later. Because of low fibrinogen concentration in canine blood (36-40 mg/dL), the sealant was not optimal for anastomosing purposes. In the second phase, the glue was prepared from 150 mL of human whole blood. Although this glue will be considered heterologous if applied to a canine case, it will be autologous if approved for clinical practice (Figure 1).

The original method used by Thorn et al.[17] to prepare the glue does not provide acceptable adhesiveness, tensile strength, and pressure bearing at the site of anastomosis. A new protocol has thus been invented to prepare a more effective sealant. By modifying and combining Thorn et al.'s and other commercial methods, finally 3 mL byproduct was prepared from 150 mL of fresh whole blood with 50-70 mg/mL fibrinogen content. Although basic principles of the procedure were according to the original method by Thorn et al.,[17] our modified method of fibrinogen precipitation was also based on high recovery rate and minimal denaturation of plasma proteins. This hybrid method consisted of multiple freeze and thawing phases, combined with ethanol, acid, and cation precipitation. Platelet-poor plasma (PPP) was initially prepared by centrifuging plasma at 10000 rpm and 4°C. PPP was then processed in a hybrid precipitation cascade. Thrombin was also harvested with sequential activation of coagulation cascade before fibrinogen precipitation (more details of the glue preparation will be discoursed in future articles after registration of the pending international patent). The concentration of growth factor in this method was about 8-12 times of that in normal plasma.[18]

After exposing of the artery and proximal and distal control, 3-5 cm of the vessels were exposed and transected by a vascular double clump. Figure 2 shows the anastomosis site and the method of glue application.

The right carotid artery was anastomosed with four 8-0 Prolene sutures in 90-degree angles. Then, 120 seconds after applying the glue, the clamps were removed and the integrity of anastomosis was evaluated. The patency of the arterial lumen was evaluated by Acland's scaling system immediately after the anastomosis.[1] Surgical incision was closed, and dogs were treated with appropriate dose of analgesics. In addition, a complete course of antibiotic therapy was commenced. After the operation, the dogs were visited by a veterinarian and the surgeon on a daily basis to check the

general condition. All dogs completed the study up to 8 postoperative weeks.

Angiographic and morphological evaluations of the vessels were performed 3 and 8 weeks after the operation. After angiography, a 20-mm biopsy including the anastomosis site was resected for histopathological examination (Figures 3 and 4). After hematoxylin and eosin staining, samples were checked under light microscope. The site of anastomosis was evaluated histologically after 3 weeks in 5 dogs and after 8 weeks in the remaining 5 (Figure 4). The vascular lumen was evaluated for thrombosis, aneurysm formation, medial necrosis, and subintimal hyperplasia. Pathological study was performed by two different pathologists. We took biopsies in 2 intervals (after 3 weeks in half of subjects and after 8 weeks in remaining) to outline early and late morphological changes in histopathology. Because after preparation of glue it was rapidly agglutinated, tensile strength could not be measured objectively.

RESULTS

After removing the vascular clamps, minimal bleeding occurred around the site of anastomosis. Evaluations by Acland's method revealed all anastomoses to be patent.[1] Figure 1 shows the prepared sealant before usage. Figure 2 depicts the gross appearance of the site of arterial repair and Figure 3 shows the angiogram.

Histopathological examination did not suggest any signs of medial necrosis, severe inflammation, or pseudo-aneurysm. In all of the 15 cases, the lumen of anastomotic site was patent (Figures 4). Subintimal hyperplasia was present in 60% of samples 3 weeks after the surgery. However, it was resolved in all cases 8 weeks post-operatively.

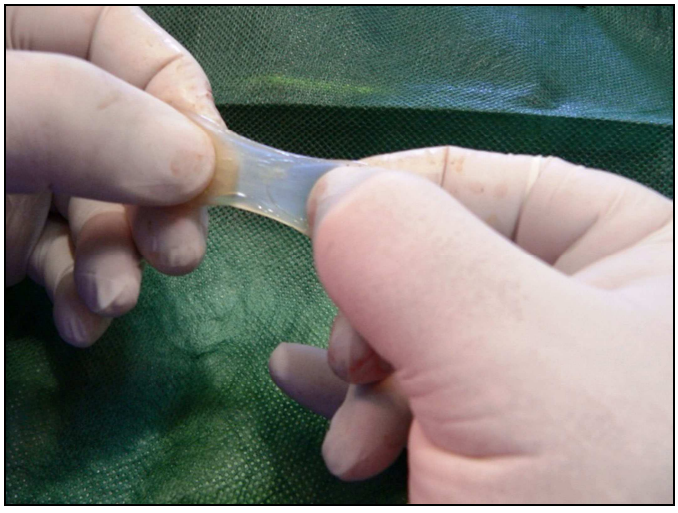

Figure 1. Tensile strength of the new fibrin glue

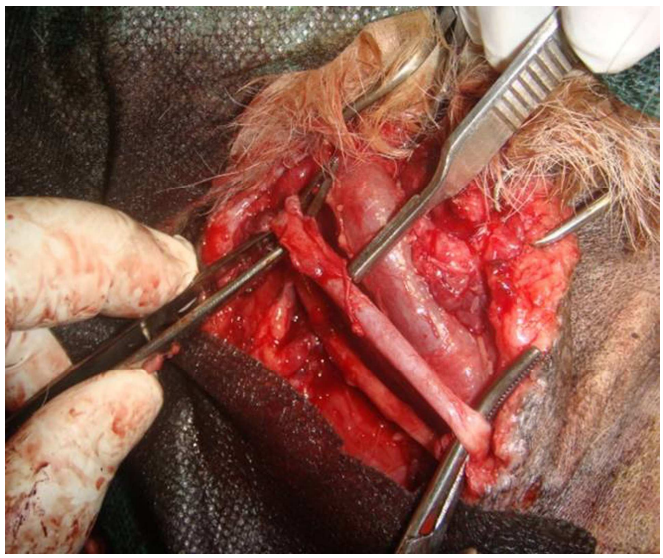

Figure 2. Application of fibrin glue on the anastomotic site

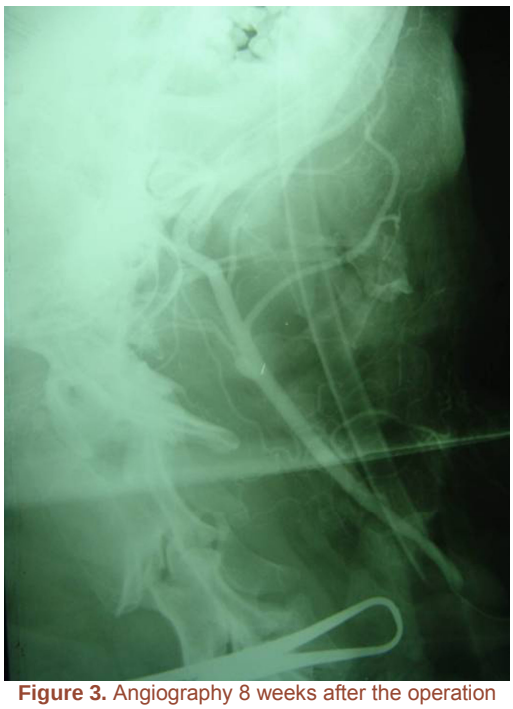

Figure 3. Angiography 8 weeks after the operation

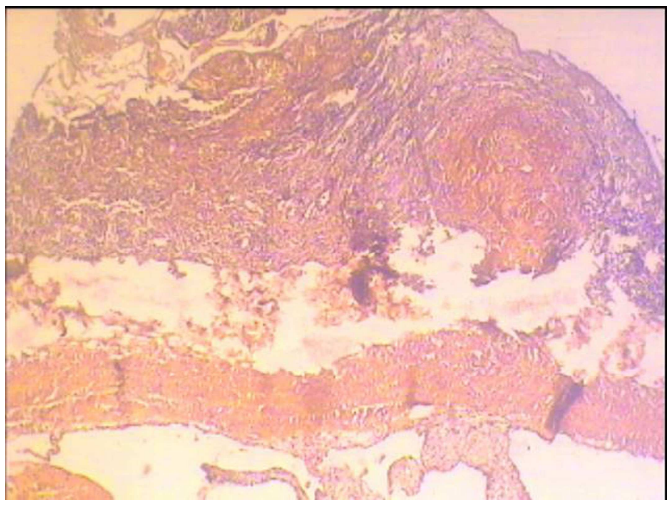

Figure 4. Microscopic view of biopsy specimen of the anastomotic site in dogs 8 weeks after the operation

DISCUSION

Finding an effective tissue sealant for end-to-end anastomosis of an artery will be a great revolution in vascular surgery. Time effectiveness, simplicity, no tissue necrosis, no foreign body reaction, and minimal trauma to vessel wall all are the potential benefits of fibrin glues. The main obstacles that hold back fibrin glue from gaining popularity are controversial primary results of fibrin glue application in suture sites and transmissible blood-borne infections by sealants derived from pooled plasma. Although this risk has recently been significantly reduced by introduction of more reliable blood processing methods, it has not been completely eliminated.[19]

The bovine thrombin, used in some available sealants, might induce hypersensitivity and antibody formation against the clotting factors and also carry the risk of prion transmission.[20] Thrombus formation in lumen of the vessel has also been reported in a few studies.[21,22]

Vascular anastomosis with single-donor fibrin glue is of utmost importance in vascular surgery. Because of difficulties in vascular surgery, particularly those seen in small caliber vessels (< 2 mm) which occur in free flaps, finger implantations, and revascularization procedures, many alternate methods have been introduced in vascular surgery.

Although many studies have suggested the application of fibrin glue for vascular anastomosis, the glue has only been clinically trialed for this purpose in 2 reports.[23,24] In one study in 1996, fibrin glue was used for digital vascular anastomosis. Using fibrin sealant reduced the number of sutures from 8-10 to only 4. In most studies, glue is employed to decrease the number of sutures and as a dressing for the anastomosis site.[28] Cho and Junior used fibrin glue for anastomosis of carotid artery in rats. As a result, they only made 6 sutures instead of the 10 stitches required in the traditional method.[1]

Until now, single-donor fibrin glue has not been used for vascular anastomosis. It has only been used as a biological dressing of suture line and hemostasis. Because of lysis of the former autologous glues within 24-48 hours, they are not suitable for vascular surgeries.[26] In our study, a new version of glue was introduced which showed satisfactory results in anastomosis of carotid artery in dogs and cats. The main advantage of the new glue was the late time of lysis. It could persist after 21 days in vivo. Another main advantage of our glue over other glues was its tensile strength.

In this study, carotid arteries of dogs were anastomosed with only 4 sutures along with our new fibrin glue. A significant reduction in the number of needed sutures was thus observed.

Cho and Junior used commercial fibrinogen (70-110 mg/mL) with 6 sutures for carotid anastomosis in rats.[1] However, the diameter of carotid artery in dogs is much larger than that of rat.

Like any other pooled plasma byproducts, the risk for viral disease transmission is still present. In addition, one of the main limitations in heterologous fibrin glue usage is hypersensitivity reactions to the sealant material particularly when bovine thrombin is used for this purpose. Another shortcoming of non-autologous glue is antibody formation against thrombin that may cause bleeding diathesis. In our study, using single-donor glue, such side effects could have been encountered.

We used hand-made fibrin glue using human blood. While this glue would be autologous for humans, it was really heterologous for the studied animals. The subintimal hyperplasia and inflammation of adventitia observed in some cases can probably be attributed to this factor.

CONCLUSIONS

Single-donor fibrin glue utilized in this study was found to produce reliable vascular anastomosis and minimize the number of sutures as well as the operation time. It did not cause hypersensitivity reactions and aneurysm formation. This method particularly decreased the risk of transmission of blood-borne infections.

REFERENCES

1. Cho AB, Junior RM. Effect of fibrin adhesive application in microvascular anastomosis: a comparative experimental study. Plast Reconstr Surg 2007; 119(1): 95-103.
2. Acland R. Technical prerequisites and tramingin microsurgery: technique of small vessel anastomosis. In: Meyer V, Black M, editors. Microsurgical procedures. Philadelphia: Saunders; 1991. p. 123-36.
3. Wolf-de Jonge IC, Beek JF, Balm R. 25 years of laser assisted vascular anastomosis (LAVA): what have we learned? Eur J Vasc Endovasc Surg 2004; 27(5): 466-76.

4. Sartorius CJ, Shapiro SA, Campbell RL, Klatte EC, Clark SA. Experimental laser-assisted end-to-side microvascular anastomosis. Microsurgery 1986; 7(2): 79-83.

5. Zeebregts CJ, Heijmen RH, van den Dungen JJ, van SR. Non-suture methods of vascular anastomosis. Br J Surg 2003; 90(3): 261-71.

6. Nakayama K, Yamamoto K, Tamiya T. A new simple apparatus for anastomosis of small vessels. Preliminary report. J Int Coll Surg 1962; 38: 12-26.

7. Wippermann J, Konstas C, Breuer M, Kosmehl H, Wahlers T, Albes JM. Long-term effects in distal coronary anastomoses using different adhesives in a porcine off-pump model. J Thorac Cardiovasc Surg 2006; 132(2): 325-31.

8. Huh JY, Choi BH, Zhu SJ, Jung JH, Kim BY, Lee SH. The effect of platelet-enriched fibrin glue on bone regeneration in autogenous bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101(4): 426-31.

9. Young JZ, Medawar PB. Fibrin suture of peripheral nerves. Lancet 1940; 1: 126-34.

10. Tidrick RT, Warner ED. Fibrin fixation of skin transplant. Surgery 1944; 15: 90-5.

11. Matras H, Jesch W, Watzek G, Dinges HP. Use of fibrin adhesives in mouth, jaw, and face surgery. Osterr Z Stomatol 1978; 75(12): 433-7.

12. Pearl RM, Wustrack KO, Harbury C, Rubenstein E, Kaplan EN. Microvascular anastomosis using a blood product sealant-adhesive. Surg Gynecol Obstet 1977; 144(2): 227-31.

13. Doillon CJ, Dion YM. Comparison of a plasma-based composite biologic sealant with fibrin glue (Tisseel) for vascular anastomoses. Surg Laparosc Endosc Percutan Tech 2004; 14(6): 335-9.

14. Kheirabadi BS, Field-Ridley A, Pearson R, MacPhee M, Drohan W, Tuthill D. Comparative study of the efficacy of the common topical hemostatic agents with fibrin sealant in a rabbit aortic anastomosis model. J Surg Res 2002; 106(1): 99-107.

15. Spotnitz WD, Prabhu R. Fibrin sealant tissue adhesive--review and update. J Long Term Eff Med Implants 2005; 15(3): 245-70.

16. Nguyen LP, Wang ZX, Molina J, Tellez A, Chemoriya T. Complications of fibrin glue in pterygium surgery with amniotic membrane transplant. Yan Ke Xue Bao 2012; 27(1): 19-24.

17. Thorn JJ, Sorensen H, Weis-Fogh U, Andersen M. Autologous fibrin glue with growth factors in reconstructive maxillofacial surgery. Int J Oral Maxillofac Surg 2004; 33(1): 95-100.

18. Foster KN, Kim H, Potter K, Matthews MR, Pressman M, Caruso DM. Acquired factor V deficiency associated with exposure to bovine thrombin in a burn patient. J Burn Care Res 2010; 31(2): 353-60.

19. Valbonesi M. Fibrin glues of human origin. Best Pract Res Clin Haematol 2006; 19(1): 191-203.

20. Leclere FM, Schoofs M, Mordon S. [Historical review and future orientations of the conventional vascular microanastomoses]. Ann Chir Plast Esthet 2011; 56(3): 232-40.

21. Dascombe WH, Dumanian G, Hong C, Heil BV, Labadie K, Hessel B, et al. Application of thrombin based fibrin glue and non-thrombin based batroxobin glue on intact human blood vessels: evidence for transmural thrombin activity. Thromb Haemost 1997; 78(2): 947-51.

22. Cho AB, Junior RM. Application of fibrin glue in microvascular anastomoses: comparative analysis with the conventional suture technique using a free flap model. Microsurgery 2008; 28(5): 367-74.

23. Han SK, Kim SW, Kim WK. Microvascular anastomosis with minimal suture and fibrin glue: experimental and clinical study. Microsurgery 1998; 18(5): 306-11.

24. Isogai N, Cooley BG, Kamiishi H. Clinical outcome of digital replantation using the fibrin glue-assisted microvascular anastomosis technique. J Hand Surg Br 1996; 21(5): 573-5.

25. Kheirabadi BS, Acheson EM, Deguzman R, Crissey JM, Delgado AV, Estep SJ, et al. The potential utility of fibrin sealant dressing in repair of vascular injury in swine. J Trauma 2007; 62(1): 94-103.

26. Buchta C, Hedrich HC, Macher M, Hocker P, Redl H. Biochemical characterization of autologous fibrin sealants produced by CryoSeal and Vivostat in comparison to the homologous fibrin sealant product Tissucol/Tisseel. Biomaterials 2005; 26(31): 6233-41.

How to cite this article: Rasti-Ardakani M, Kalantar-Hormozi A, Rasti-Ardakani J, Hosein Davarpanah Jazi AH, Shayesteh Moghadam A. Introduction of a potent single-donor fibrin glue for vascular anastomosis: An animal study. J Res Med Sci 2012; 17(5): 461-5.

Source of Support: Nil, **Conflict of Interest:** None declared.