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Organic Glues or Fibrin Glues from Pooled Plasma: Efficacy, Safety and Potential as Scaffold Delivery Systems

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ABSTRACT - Since 1976, fibrin glues have been attracting medical interest, spreading from their initial use as a hemostatic agent in cardiovascular surgery to other fields of surgery. Studies have compared the efficacy of fibrin glues vs sutures in surgery. However, few comparisons have been made of the efficacy and safety of the different fibrin glues commercially available. Recently, fibrin glues have been tested as a scaffold delivery system for various substances inside the body (drugs, growth factors, stem cells). The infectious risk (viruses, new germs) of this blood-derived product was also studied in assays on viral inactivation methods. The development of autologous fibrin glues offers a solution to the problem of infectious risk. This review examines the current state of knowledge on the efficacy, safety and future potential of fibrin glues.

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INTRODUCTION

Fibrin has been described as a hemostatic agent for more than 100 years, but it has only been used in humans since the 1960s. It was initially marketed in the late 1970s to promote hemostasis as a suture support in surgery, or to provide wound sealing when bleeding cannot be controlled using conventional methods. Fibrin glues are mainly extracted from pooled plasma and contain different amounts of purified and virally inactivated human proteins: fibrinogen, thrombin, factor XIII (F XIII), anti-fibrinolytic agents and calcium chloride (1). The glues mimic the last step of the physiologic blood coagulation cascade to form a fibrin clot, hence providing early hemostasis on the treated area.

Fibrin glues are usually prepared from whole blood by cryoprecipitation, followed by two-step viral inactivation/removal. Because fibrin glues from pooled plasma are plasma-derived products, their use exposes the receiver to the risk of pathogen transmission from the donor blood. Over the last thirty years, various studies have assessed the efficacy and the safety of fibrin glues.

Prospective applications for autologous fibrin glues include replacing fibrin glues from pooled

plasma, since autologous glues offer advantages like no risk of infection or hypersensitivity.

This review highlights, in addition to the conventional applications, potential applications of the glues in areas such as scaffold delivery of drugs and other substances.

ROLE OF FIBRIN GLUES IN THE COAGULATION CASCADE

Coagulation cascade

Physiologic blood clotting leads to synthesis of fibrinogen, a glycoprotein containing three dimers of polypeptides (2 $A\alpha$, 2 $B\beta$ et 2 γ) linked to each other by disulfide bonds. Fibrinogen is cleaved by thrombin to release fibrin and two fibrinopeptides A and B (FpA, FpB). Then, the soluble fibrin monomers polymerize spontaneously thanks to hydrogen bonds, forming an insoluble and stable fibrin polymer. F XIII (zymogen activated by thrombin in the presence of calcium into a transglutaminase) catalyzes the formation of covalent bonds between fibrin chains, to stabilize

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and to strengthen the mechanical stability of the fibrin clot and to enhance its resistance to clot lysis. During fibrinolysis, plasmin cleaves fibrin into 5 fibrin degradation products (FDPs). The mechanism of the action of fibrin glues is now well established (1,2). Fibrin glues echo the last step of blood clotting, operating separately from the physiologic coagulation mechanism. This

enables fibrin glues to be used in treating patients with coagulopathy (3-7).

A double-syringe system (fitted with a cannula), containing both the fibrinogen component and the thrombin component separated into two different channels, is used to apply the glue: the two components are externally mixed at the tip of the cannula, on the wound (8).

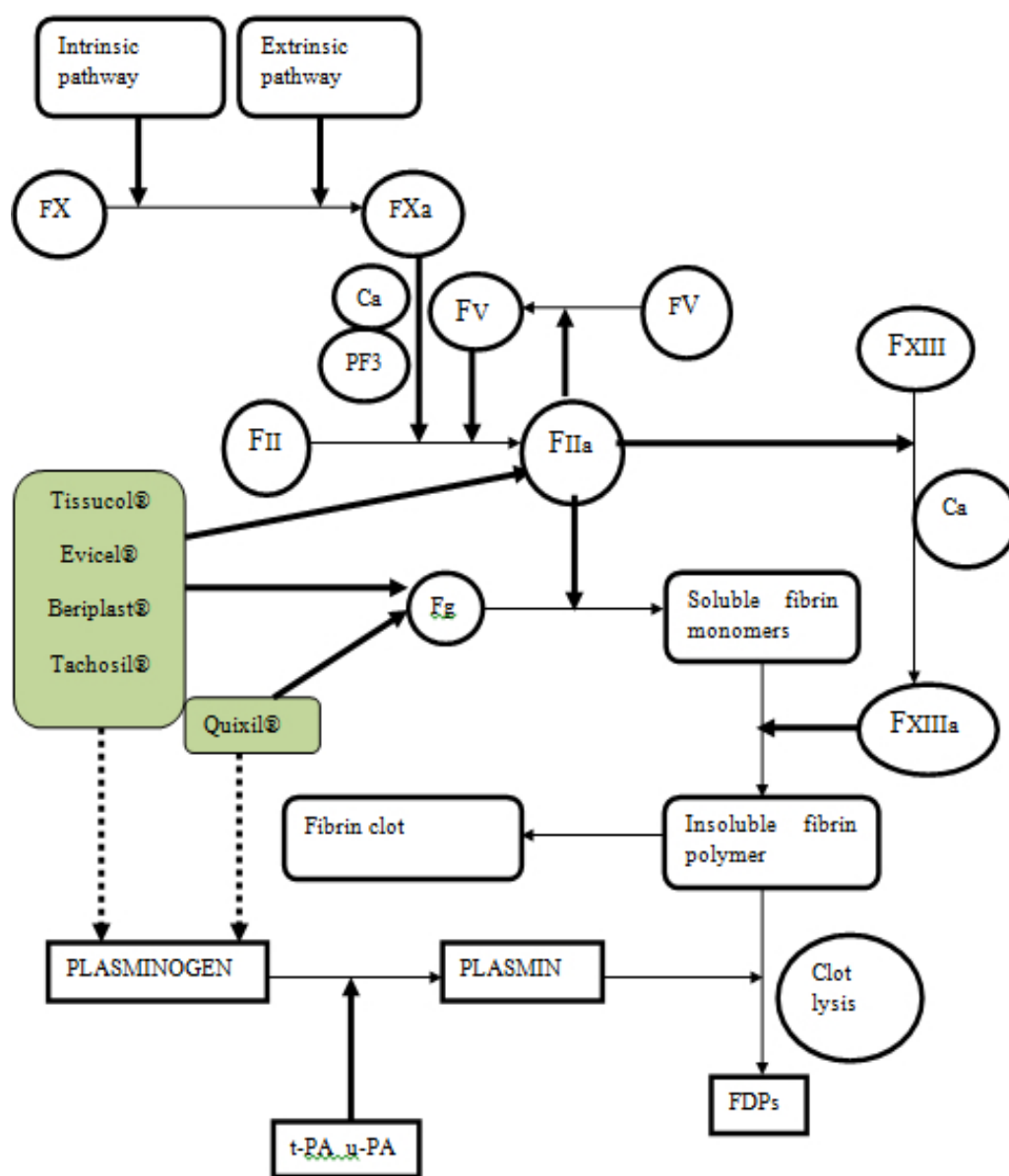


Figure 1. Coagulation cascade and fibrin glue targets. FX = factor X; FXa = activated factor X; Ca = ionized calcium; FV = factor V; FVa = activated factor V; FII = thrombin; FIIa = activated thrombin; Fg = fibrinogen; FXIII = factor XIII; FXIIIa = activated factor XIII; FDPs = fibrin degradation products; t-PA = tissue plasminogen activator; u-PA = urokinase plasminogen activator; PF3= platelet factor 3. Full arrows in bold type: activation; Dotted arrows in bold type: inhibition

Thrombin (dissolved in a calcium chloride solution) converts fibrinogen into fibrin monomers, to form unstable fibrin clots. F XIIIa catalyzes cross-linking between glutamyl and lysyl residues of adjacent fibrin fibers: it cross-links both the γ -chains (fast reaction in the presence of low concentration of F XIII = 0.5 U/mL) and the α -chains (slow reaction in the presence of high concentration of F XIII = 10 U/mL) of fibrin. The cross-linking by F XIIIa makes the clot mechanically stable. Polymerization is achieved within two hours. At the same time, F XIII is effective in a variety of ways: acceleration of wound healing promotes migration and proliferation of fibroblasts into the clot, resistance to lysis by plasmin creates covalent bonds between fibrin molecules and α 2-antiplasmin. Clot lysis is achieved within two weeks, the time it takes for the anti-fibrinolytic agent to diffuse out of the fibrin clot.

Five products are commercially available in France: Evicel[®], Quixil[®], Tissucol[®], Beriplast[®] and Tachosil[®]. All of these glues contain thrombin and fibrinogen from pooled plasma, but their final qualitative and quantitative composition differs, so they cannot be considered generic products (1, 9).

Thus, commercially available fibrin glues differ in the quantities of F XIII they contain. When the role of this factor in glue efficacy was assessed (2), Beriplast[®], with the highest F XIII content (40-80 U/mL), showed a rate of complete γ - γ chains cross-linking within one minute that was significantly higher ($p < 0.05$) than the others. Late hemostasis (seven days after application of the fibrin sealant) was achieved in

100% of cases with Beriplast[®] but in only 27 % of cases using fibrin glue without F XIII ($p < 0.01$). This finding shows that F XIII improves the quality of fibrin glues by i) making fibrin clots more resistant to lysis by plasmin (covalent bond between F XIII and α 2-antiplasmin); ii) increasing the tensile strength of the clot up to fivefold; iii) reducing blood loss; iv) decreasing the need for fibrin reapplication (only 20 % compared with 46.7 % for fibrin glues without F XIII); v) reducing postoperative adhesions -correlated with premature clot lysis- to 46.7 % for fibrin sealant containing F XIII, as compared with 80 % for sealant without F XIII ($p < 0.06$).

Anti-fibrinolytic agents (employed to prevent clot lysis before use and to preserve fibrin clots until wound healing) used in fibrin sealants vary: bovine aprotinin (serine protease inhibitor) in Tissucol[®] and Beriplast[®], tranexamic acid in Quixil[®], no anti-fibrinolytic agent in Tachosil[®]. The need to include an anti-fibrinolytic agent in fibrin glues has been debated in the literature (10). Thus, unlike its predecessors TachoComb[®] (containing bovine aprotinin and bovine thrombin) and TachoComb H[®] (containing bovine aprotinin and human thrombin), Tachosil[®] is free of bovine components. Indeed, aprotinin has been shown not to affect fibrinolysis: the time required for hemostasis when fibrin glue is administered with heparin increases to 75 % ($p = 0.005$) for Tachosil[®] and to 80 % for TachoComb[®] ($p = 0.001$). Furthermore, Tachosil[®] shows the same hemostatic efficacy as TachoComb H[®], even in hyperfibrinolysis conditions (11).

PREPARATION OF FIBRIN GLUES

Table 1. Composition of fibrin sealants

	TISSUCOL [®]	BERIPLAST [®]	QUIXIL [®]	TACHOSIL [®]	EVICEL [®]
Plasma proteins	100-130 mg/mL	65-115mg/mL	60-80 mg/mL	NC (equine collage)	80-120 mg/mL
Fibrinogen	70-110mg/mL	90 mg/mL	40-60 mg/mL	5.5 mg/cm ²	55-85mg/mL
Thrombin	500 UI/mL	500 UI/mL	1000 UI/mL	2 UI/cm ²	800-1200 UI/mL
Factor XIIIa	10-50 UI/mL	40-80 UI/mL	-	-	91 UI/mL
Fibronectin	5.5 mg/mL	-	40-60 mg/mL	-	NC
Plasminogen	0.08 mg/mL	-	-	-	-
Anti-fibrinolytic agent	Bovine aprotinin 3000 UI/mL	Bovine aprotinin 1000 UI/mL	Tranexamic acid 100 mg/mL	-	-

Table 2. Data on fibrin glues from pooled plasma

	EVICEL [®] (Johnson and Johnson)	TISSUCOL [®] (Baxter)	BERIPLAST [®] (ZLB Behring)	QUIXIL [®] (Johnson and Johnson)	TACHOSIL [®] (Nycomed)
Presentation	Frozen components for glue solution 1/2/5 mL	Lyophilized component for reconstitution 1/2/5 mL	Lyophilized component for reconstitution 0.5/1/3 mL	Frozen components for glue solution 2/5 mL	Sponge 23/45 cm ²
Indications	Adjunct to hemostasis in surgery when conventional surgical techniques are inadequate or impractical		Reinforcement of sutures Tissue gluing	Adjunct to hemostasis in surgery when conventional surgical techniques are inadequate or impractical	
Dosage	Liq : NC Sp: 20-100 cm ²	Liq : 10 cm ² Sp: 25-100 cm ²	Liq : NC Sp: 50-100 cm ²	Liq : 10 cm ² Sp: 20-40 cm ²	23 cm ² 45 cm ²
Conservation	2 years (- 18°C)	2 years (2-8°C)	2 years (2-8°C)	2 years (- 18°C)	3 years (25°C)
Stability	24 h (25°C)	4 h (25°C)	24 h (25°C)	24 h (25°C)	3 years (25°C)
Stability after reconstitution	30 days (2-8°C)	4h	8-24h	30 days (2-8°C)	-
Reconstitution	Thawing 1 day (2-8°C) 1 h (20-25°C) 10 min (37°C) Draw into applicator	Reconstitute Warm 40 min (37°C) Draw into applicator	Reconstitute Warm 15 min (37°C) Draw into applicator	Thawing Draw into applicator	Ready for use
Resorption	-	2 weeks	2 weeks	2 weeks	24 weeks
Status	PDPs: traceability and pharmacovigilance				

PDPs = plasma-derived products; Liq = liquid; Sp = spray.

Table 2 summarizes features of the fibrin glues commercially available. However, these features can differ from country to country for the same product (12). Quixil[®] is very handy and easy to use: it is quickly prepared (5-7 min) and does not require warming, so Quixil[®] is very useful when it is decided at the very last moment to apply fibrin glue. In addition, this product can be stored for one month at 2-8°C if there is a cold chain break. Quixil[®] possesses a triple lumen catheter which makes it handier than other fibrin glues: the fibrin component and the thrombin component remain separate right up to the tip of the catheter, only meeting on the wound. This precludes clots forming in the catheter, so fibrin glue loss is reduced. Finally, this product enables surgeons to switch between spraying and dripping without changing devices (9). However, there is one main drawback to Quixil[®]: it remains transparent

before and after coagulation. There is no visible change, therefore, to confirm that the glue is acting (unlike other fibrin glues that become opaque after coagulation), although the transparency makes it possible to preserve the visibility of underlying tissues.

Fibrin sealant may be denatured after exposure to solutions containing ethanol, iodine or heavy metals. For this reason, the European Medicines Evaluation Agency (EMA) recommends in The Guideline on The Clinical Investigation of Plasma Derived Fibrin Sealant/Haemostatic Products that the use of these antiseptic solutions before applying the glue be avoided (13).

Fibrin sealant manufacture

Methods of manufacturing fibrin glues differ: Schwartz method for Tissucol[®] and Beriplast[®]

(production of purified fibrinogen), Martinowitz method for Quixil[®] (production of biologically active cross-linked fibrinogen-fibronectin multimers). Fibrinogen-fibronectin multimers enable the fibrin clot to increase its strength when binding to collagen, thus enhancing adhesion of the clot to the extracellular matrix (14).

The fibrinogen concentrate is obtained by centrifugation from whole blood, followed by cryoprecipitation or chemical precipitation with ethanol, polyethylene glycol (PEG) or ammonium sulfate.

Cryoprecipitation is the most common method of making fibrin glue and involves several freeze-thaw cycles: freezing blood between -20°C and -80°C for 1-24 hours and then thawing blood at 4°C for 12 hours. Fibrinogen is then removed by centrifugation of plasma. The quantity of fibrinogen obtained ranges from 20 to 40 mg/mL; however, this method involves numerous variables (freezing and thawing conditions, number of freeze-thaw cycles), which impedes reproducibility.

Chemical methods are faster (single precipitation step) and yield higher quantities of fibrinogen (30-50 mg/mL), but the purity of the product is an issue (15). Using ethanol extraction leads to premature clotting of the fibrinogen due to the high concentration of alcohol in the end product; ethanol also decreases F XIII activity, which reduces the tensile strength of the clot (16, 17). Ammonium sulfate precipitation increases the concentration of albumin in the glue, which can interfere with clotting (18); but this ammonium sulfate can precipitate a higher quantity of fibrinogen, leading to higher tensile strength (41 g/cm² 10 min after thrombin addition). Using PEG to harvest fibrinogen requires numerous steps and makes the glue less clottable (19). Ethanol and PEG are therefore less efficient than ammonium sulfate for harvesting fibrinogen, giving ammonium sulfate the edge over other chemical methods (20).

The thrombin component is manufactured separately from the fibrinogen component: a pre-

purified human prothrombin is activated into thrombin and then undergoes purification by chromatography (21).

Plasma-derived products are potential virus-transmitters. To ensure viral safety, therefore, certain measures need to be taken with respect to this residual risk when manufacturing fibrin glues. First, blood donors are carefully selected. Then, all blood donations are screened for HIV-1, HIV-2, HBV, HCV and parvovirus B19. Finally, to decrease the risk of prion transmission, bovine aprotinin has been replaced by tranexamic acid in Quixil[®] (9).

EMA recommends that plasma-derived products undergo two complementary validated processes for virus inactivation/removal, to increase efficacy against unknown viral pathogens. One of these inactivation steps should be effective even against non-enveloped viruses. Quixil[®] undergoes two different methods of viral inactivation, and thus meets the EMA guidelines. However, with both Tissucol[®] and Beriplast[®] the two steps of viral inactivation are identical instead of complementary in their mode of action, and therefore these products do not meet the EMA guidelines. Nevertheless, the risk of virus transmission for Tissucol[®] and Beriplast[®] is assessed as low, with a good margin of safety. From 1991 to 2000 (Behring data from post-launch period), 5.6 million doses of Beriplast[®] were applied and no cases of HIV or HBV transmission were registered (2).

The safety of plasma-derived products with respect to HIV, HBV and HCV is now well established, even when a single mode of virus inactivation is used. On the other hand, several reported cases of parvovirus B19 transmission have been linked to coagulation factors or fibrin sealant (22, 23). NAT (Nucleic acid Amplification Testing), a new screening method to eliminate viruses, limits the amount of parvovirus B19 to below 10⁵ geq/mL, but risk of infection remains.

Table 3. Viral inactivation/removal methods

	Fibrinogen	Thrombin
QUIXIL [®] EVICEL [®]	Solvent detergent treatment (1% TnBP, 1% Triton X-100) for 4h at 30°C Pasteurization for 10h at 60°C	Solvent detergent treatment (1% TnBP, 1% Triton X-100) for 6h at 26°C Nanofiltration (28 µ)
TISSUCOL [®] BERIPLAST [®]	Two-step vapor heating at 60°C and 80°C 2 pasteurizations	

ASSAYS PERFORMED

Table 4. Comparison of fibrin glue efficacy

	Adhesive strength in vitro	Efficacy in vivo (hemostasis)	
		Early	Late
BERIPLAST®	+++	+++	+++
TISSUCOL®	+	+++	+++
QUIXIL®	+++	+	+

Criteria to be met in fibrin glues include achieving fast hemostasis, high clot strength and elasticity and good clot adhesion to the wound (9).

Fibrin sealants are used as hemostatic agents in several fields of surgery, including cardiovascular surgery (24-26), urology (27), otolaryngology (28, 29), neurosurgery (30-33), orthopedic surgery (34, 35), gastro-intestinal surgery (although benefits are not well established) (36-38), thoracic surgery (39-41) (for air leak sealing). For tissue gluing and tissue attachment, fibrin glues are not an adequate substitute for sutures, as their mechanical strength is low (42). Tissucol® and Beriplast® have been shown to achieve hemostasis in 100% of cases after the first application, whereas hemostasis is achieved in 30% of cases with Quixil® (1).

Retrospective monocentric studies are usually undertaken on small cohorts and present a varying standard of evidence (low for Beriplast®, good for Tissucol®). To date, there have been no comparative trials involving all commercially available fibrin glues (43).

Quixil®

Because of its low F XIII activity (< 1UI/mL) and fibrinogen content, Quixil® presents a significantly greater elongation ($p < 0.008$) compared with Beriplast® and Tissucol®. Moreover, the higher concentration of thrombin in Quixil® can contribute to rapid hemostasis. However, Quixil® does not possess good hemostatic properties, consequently requiring application in greater volume (1.75 mL +/- 0.39 mL versus 1.2 mL +/- 0.00 mL with Beriplast®) to obtain similar results. In animal models, a second application was required in 70% of animals ($p < 0.05$), whereas complete hemostasis was rapidly achieved in 100% of animals after the first Beriplast® spraying (1). Moreover, a zero rate of late hemostasis was found with Quixil®, as opposed to 100% with Beriplast® ($p < 0.05$). Generally,

fibrin glues with poor F XIII activity lead to weak fibrin clots and are associated with premature clot lysis, thus putting the patient at higher risk of hemorrhage. In addition, the fibrin clot obtained with Quixil® disintegrated within one week, exposing fragile tissue in its granulation phase (1).

Another study carried out with animal models assessed time to hemostasis and blood loss: Quixil® reduced time to hemostasis and blood loss, as well as requiring less volume than the other fibrin sealants. Indeed, blood loss was measured at 372 mL and lasted 372 seconds without any fibrin glue; after applying Quixil® blood loss was less than 1 mL and lasted only 25 seconds (44).

Basically, clinical trials have focused on orthopedic and hepatic surgery, although Quixil® is licensed for all types of surgery except neurosurgery (44). Thus, Quixil® was tested in orthopedic surgery (45) and revealed blood loss reduction 12 hours post-surgery (184.5 +/- 28.9 mL versus 408.3 +/- 54.6 mL in control group, $p = 0.002$).

Finally, one study found that Quixil® decreases time to hemostasis in vascular surgery (2.5 min versus 17 min in control group, $p = 0.001$) (46).

Tissucol®

The tensile strength obtained with Tissucol® is significantly lower than with other fibrin glues (0.008-0.012 N versus 0.24-0.37 N, $p < 0.006$) (1). However, Tissucol® scores well on early and late hemostasis (100%) and allows a more homogenous application of the fibrin glue layer than Beriplast®.

Several studies involving Tissucol® in numerous fields of surgery (hepatic, urological, gynecological, thoracic, vascular, ENT, neurological) have been performed.

A clinical trial assessed Tissucol® efficacy in cardiopulmonary surgery and found the fibrin

glue to be more effective than conventional surgical patient care in achieving fast hemostasis. Indeed, Tissucol[®] was used with 92.6% success to seal a bleeding wound within 5 min, versus 12.4% success using conventional methods ($p < 0.001$). Furthermore, the fibrin glue used as second treatment allowed bleeding control in 82.0% of cases, and postoperative blood loss was significantly lower in the fibrin sealant group ($p < 0.05$) (47).

In six laparoscopic spleen-preserving procedures, Tissucol[®] eliminated both the need for laparotomy and postoperative bleeding (48).

Similarly, in the treatment of primitive and secondary abdominal relapsed fistulas, Tissucol[®] registered a high success rate and acted rapidly (49).

Beriplast[®]

Few of the clinical studies available specifically consider Beriplast, most being based on data for Tissucol, whose composition is similar (43).

Beriplast[®] is recognized for use in several types of surgery as an effective hemostatic agent (neurosurgery, aerostasis) (50). As the end of bleeding is correlated with immediate hemostasis, the best results were found with Beriplast[®]: in a comparative study, optimum F XIII activity was reported to be about 60 UI/mL or more, in order to obtain the fastest hemostasis. Beriplast[®] achieved the best early hemostasis (clotting obtained in 100% of cases after first application) and the lowest blood loss (1).

However, this study highlighted the need for large quantities of fibrin to enhance wound healing. It should be noted that excessive fibrin concentration prevents neutrophils from migrating into the clot, an important step in wound healing. In the same way, excessive thrombin activity (> 1000 UI/mL) could activate intravascular clotting. It is therefore essential to apply well-homogenized fibrin glue in a thin layer on the wound.

Tachosil[®]

Fifteen years of experience with TachoComb[®] can be exploited in Europe. Assays were mainly undertaken in hepatic surgery, but Tachosil[®] is licensed for all branches of surgery. Indeed, Tachosil[®] is effective in multiple indications, as shown by randomized controlled trials concerning hepatic surgery, thoracic surgery and urology (51). Moreover this product is very cost-effective compared with other fibrin sealants.

Evicel[®]

A comparative study of Evicel[®] versus Tisseel[®] was carried out with regard to mechanical, kinetic and biochemical properties. Evicel[®] led to significantly higher tensile strength of the fibrin clot than Tisseel[®] ($p < 0.05$), within a shorter period of time, and presented greater resilience to stretching. These results were attributed to the higher F XIII content in Evicel[®] compared with Tisseel[®] (52). When Evicel[®] was assessed in vascular surgery, it reduced the time to hemostasis, allowing hemostasis to be achieved within four min in 85% of patients versus 39 % of patients using manual compression ($p < 0.001$) (53). Evicel[®] was also tested in rhinoplasty: bruising and swelling significantly decreased after its application on a lateral osteotomy site on postoperative days 1, 7 and 21 (54).

Methods of application

Fibrin glue can be applied in two different ways: dripping and spraying. However, spraying leads to better hemostasis than dripping, as well as to lower blood loss: only 7 mL +/- 16 mL with spraying versus 110 +/- 160 mL with dripping. The new rub-and-spray application method was assessed for the treatment of acute aorta dissection (high-risk surgery due to systematic heparinization and hypothermic cardiopulmonary bypass) and obtained the most effective hemostasis with the strongest sealing and the lowest postoperative blood loss. Using this method, a fibrinogen solution is first rubbed on the wound and then fibrinogen and thrombin solutions are simultaneously sprayed on (55).

Salmon fibrin glue

Fibrin glue prepared from the blood of farmed Atlantic salmon was assessed and found to significantly decrease the risk of infection by viruses, bacteria or prions compared with fibrin glue prepared with human or bovine proteins. This strong barrier to trans-species infections is due to the much lower ambient temperature and the remote phylogeny of salmon. The study reported similar structures and properties for fibrinogen and thrombin from salmon and from human fibrin clots; the mechanism of the clotting cascade is also similar to that in mammals. Moreover, salmon fibrin sealant achieved greater hemostasis, forming clots insensitive to changes in pH and ionic strength. No immune reactivity was developed with salmon glues (56).

Scaffold for drug delivery system and tissue engineering

Drug delivery system

Fibrin glues have been investigated as drug delivery systems. As they undergo slow lysis, they can be used as a scaffold for several molecules.

Growth factors

Fibrin sealant can be used as a binding reservoir for growth factors VEGF (Vascular Endothelial Growth Factor), TGF- β 1 (Transforming Growth Factor), IGF (Insulin Growth Factor) and bFGF (basic Fibroblastic Growth Factor) (57-60). The kinetics of the release of these growth factors from fibrin glue may increase vascularization of subcutaneous tissues. In a study, interaction between TGF- β 1 and fibrin was observed: indeed, TGF- β 1 release from the gel decreased with increasing fibrinogen content. After being released from platelets, TGF- β 1 appears to be temporarily stored in the fibrin clot, thus prolonging TGF- β 1 activity in the target zone. Thus, several assays show improved tissue engineering using fibrin glue as a scaffold for growth factor release (61-64).

bFGF can induce the migration and proliferation of endothelial cells (enhanced angiogenesis) and smooth muscle cells (myocardial tissue repair). Application of fibrin glue containing this growth factor to transmyocardial tissue in order to improve angiogenesis and cardiac perfusion was assessed in animal models. Direct injection of bFGF into transmyocardial tissue increased total vascular density by 40%, but a two-fold increase in the number of large vessels was observed. Hence bFGF alone appears to increase vessel size but not the total number of vessels. When administered via fibrin glue, bFGF increases total vascular density by 28%, with a 59% increase in the number of large vessels; consequently, fibrin sealant containing bFGF could induce arteriogenesis (63).

Anesthetic drugs

Fibrin glues containing lidocaine were administered in order to reduce postoperative pain. After breast augmentation, fibrin glue containing lidocaine was injected into the subpectoral pocket: postoperative pain was significantly lower ($p < 0.05$) compared with patients receiving lidocaine or fibrin glue alone (65, 66). Similarly, fibrin glue containing

lidocaine was applied on the tonsillar fossae after tonsillectomy to evaluate its analgesic effects: time to normal eating was shortened to 2.83 days (versus 3.178 days for patients receiving regular fibrin glue) and time of analgesic administration was decreased to 2.88 days (4.91 days for patients receiving regular fibrin glue) (67).

Antibiotics

Fibrin sealants have also been used as a drug delivery system for antibiotics. In a study, vancomycin was incorporated into the fibrinogen component and applied to vascular Dacron grafts in rats. Local vancomycin concentration in the target area was found higher using fibrin glue as a carrier, compared with intravenous injection of vancomycin (89.58 μ .h/g with a targeting index of 12.11 versus 7.40 μ .h/g). *S. aureus* was injected into Dacron grafts on rats: none of the six grafts treated with fibrin glue containing vancomycin became infected, whereas two out of the six grafts treated by vancomycin IV injection became infected (68).

A case report on a patient suffering from acute endocarditis revealed the efficacy of vancomycin incorporated into Beriplast® (0.5 g of vancomycin/3 ml of fibrin glue) in vascular graft surgery (patch composed of Dacron graft and pericardium using fibrin glue containing vancomycin): 11 months after the surgery no prosthetic valve endocarditis had been reported (69).

Other antibiotics were tested with fibrin glue: a concentration of 400 μ g of teicoplanin, cephalothin or gentamicin was incorporated into 1 g of fibrin glue and then added to a 500 IU/ml thrombin solution. Antibiotics were released within 96 hours, thereby initiating antibacterial activity during this time, which suggests that this delivery system offers prevention and treatment benefits in ocular surgery (70).

Chemotherapeutic agents

Treatment of patients suffering from cancer by fibrin glues containing chemotherapeutic agents led to increased agent concentration in tumors and reduction of adverse effects.

First, an in vitro study assessed the local drug delivery of doxorubicin from Beriplast® (71). Three groups were treated with doxorubicin: doxorubicin with solution, doxorubicin incorporated in fibrin glue and doxorubicin incorporated in fibrin glue containing sodium alginate. In vitro release of this anti-cancer molecule was found to take respectively 3.7 h, 8.7

h and 81 h. Since prolonged release of doxorubicin was better with fibrin glue containing sodium alginate, an in vivo study was undertaken on an AH60C tumor in the rat back. Fibrin glue containing 6 mg of doxorubicin and 2.5 mg of alginate sodium was applied, resulting in an extremely high concentration of doxorubicin in the tumor extracellular fluid (concentration inversely correlated with tumor volume, $r = 0.882$), while the plasma concentration remained low.

Second, a study compared the release properties of several anti-cancer drugs from an autologous fibrin glue both containing and not containing aprotinin (72). Fluorouracil, Tegafur and Mitomycin C mixed with fibrin glue (with or without aprotinin) were quickly released (steady state level after 1-2 hours), suggesting the lack of interaction between these molecules and autologous fibrin glue. However, when Etoposid and Doxorubicin were mixed with fibrin glue containing aprotinin, their concentration gradually increased, peaked after 24 hours, then gradually decreased. Thus this study proves that aprotinin can be valuable in controlling the speed of fibrin glue lysis. The findings of this study show that the release properties of anti-cancer drugs differ according to hydrophobicity: they need to be lipophilic to achieve sustained release from fibrin glue.

Stem cells

Mesenchymal stem cells (MSC) from bone marrow can be mixed with fibrin glues in order to regenerate or repair tissues (especially bone tissue). Fibrin glue is a suitable vehicle for mesenchymal stem cell transplantation: it promotes angiogenesis and its F XIII content enhances the migration and proliferation of stem cells (73).

Platelet-rich fibrin (PRF)

PRF is extracted by centrifugation from whole blood without anticoagulants, thus creating a clot in the middle of the tube containing principally fibrin, platelets and leukocytes. PRF can be used directly as a healing biomaterial; alternatively, it can be used as a biological connector between grafted elements (after being cut and mixed with grafts) and will induce neo-angiogenesis in the graft. Biological membranes can be produced from PRF after withdrawing and compressing the serum with a piston (tympanoplasty).

In chondrogenesis, PRF is also a biological scaffold which has been tested as an MSC carrier:

α -granules contained in platelets release growth factors (TGF- β 1 and IGF-1) that enhance cartilage regeneration. A clinical trial was carried out on five patients suffering from cartilage lesion of the femoral condyle: bone marrow was extracted from the iliac crest, then isolated nucleated cells were cultured in fetal bovine serum and mixed with PRF. After curettage of the sclerotic bone, the mixture of MSC-PRF was applied on the injured area. This technique resulted in major improvement in symptoms and knee function within 6-12 months ($p < 0.05$) for all the patients, all of whom were able to regain their normal sports fitness level within 9 months (64). However, this study included only five young patients (< 37 years old), which may influence outcomes, since cartilage repair mainly depends on the metabolic activity of cells and thus is age-dependent.

The potential of PRF as a carrier for adipocyte grafts in facial liposstructure was evaluated via the stimulation of proliferation of pre-adipocyte cells by a fibrin matrix and the stimulation of adipocyte graft healing by platelet cytokines (74). All 32 patients treated by this technique were satisfied with the end result and no additional adipocyte graft was needed. Indeed, there was no notable resorption even with an HIV-seropositive patient; however, it is difficult to evaluate this criterion without comparative pre- and postoperative magnetic resonance imaging (MRI). Moreover, there was no control group treated without PRF, which makes results difficult to interpret. In conventional facial liposstructure, major edema persists in 10% of patients and major bruises in 6% of patients after 3 and 4 weeks respectively (75), whereas the study reported no cases of edema or bruises. Indeed, angiogenesis is enhanced by grafts containing PRF due to increased vascular and lymphatic drainage, with consequently lower risks of edema or bruises.

In case of stress urinary incontinence, fibrin glue containing muscle-derived stem cells (MDSC) is able to promote urethral sphincter restoration. Fibrin can induce angiogenesis, promote cell attachment and proliferation. Transplantation of fibrin sealant mixed with MDSC resulted in improvement of the stem cells' life-span ($p < 0.05$), increased thickness of urethral muscle and higher microvessel density ($p < 0.01$), compared with a group receiving MDSC alone. This suggests that fibrin glue may enhance the differentiation of MDSC towards that of well-differentiated muscle cells (76).

Hemostasis and tissue adhesive

Fibrin glue has the advantage of reducing time to hemostasis in vascular surgery compared with conventional manual compression (77).

In the surgical treatment of inguinal repair by Lichtenstein technique (with sutures), complications frequently reported are chronic pain and high recurrence rate. The use of fibrin glue as a substitute for the previous technique led to similar outcomes with regard to mean operation time, mean length of postoperative hospitalization and mean time to return to work. However, postoperative incidence of chronic pain, inflammation and hematoma were lower when using fibrin glue ($p < 0.001$) (78-81). Indeed, several months after surgery, none of the patients treated with fibrin glue had developed a recurrence and 80% reported complete absence of chronic pain ($p < 0.05$) (79, 81).

Fibrin sealants have been assessed in the treatment of acute ruptures of the Achilles tendon: all patients thus treated were reported cured within five months. In the long term, no pain, no re-rupture and no loss of strength of the Achilles tendon was observed (34).

Fibrin sealants have also proved valuable in obstetrical gynecology. The iatrogenic preterm premature rupture of the fetal membrane (iPPROM) can be due to invasive diagnosis (fetoscopy or amniocentesis). A comparative study was undertaken involving Tissucol[®] versus five synthetic biocompatible glues for the treatment of iPPROM. It was observed that only Tissucol[®] and cPEG (catechol-functionalized poly(ethylene glycol)) were able to achieve a strong bonding to fetal membrane (showing a continuous layer between glue and fetal tissue) and were demonstrated to have no in vitro toxicity after 24 hours. Consequently, both can be considered to repair fetal membranes without being toxic for the fetus (82). Puncture of the fetal membrane can also result in a decrease of its tensile strength, which application of fibrin glue can restore; moreover, it may improve the structural and functional integrity of ruptured membrane (83).

Finally, the treatment of anal fistula by injection of fibrin glue can eradicate the risk of fecal incontinence observed in treatment by fistulotomy (84). However, fibrin sealants are ineffective in the treatment of complex anal fistula: only 10% of patients were cured after 12 months (85), although fibrin glues proved very effective in treating patients suffering from Crohn's disease (86). For the management of

duodenal perforation in rats, assays revealed that fibrin glues were no more effective than the conventional suture technique (87).

Fibrin glue toxicity***Bovine aprotinin hypersensitivity***

It has been shown that aprotinin does not improve the hemostasis performance of fibrin glue (88). The study compared Tisseel[®] to a fibrin glue not containing an anti-fibrinolytic agent, and observed a reduction in early hemostasis, higher blood loss and lower survival for Tisseel[®]. Blood loss in the group receiving fibrin glue without an anti-fibrinolytic agent was 1.9 +/- 1.9 mL and survival rate was 75% (6/8), while in the group receiving Tisseel[®], mean blood loss was 30 +/- 6.0 mL and survival rate was 43% (3/7) ($p < 0.01$). Thus, the legitimacy of adding an anti-fibrinolytic agent to fibrin can be questioned, particularly since aprotinin can cause hypersensitivity reactions. Indeed, bovine aprotinin contained in fibrin glue is responsible for the production of anti-bovine Factor V antibodies that may cross-react with Human Factor V and could lead to severe bleeding. Bovine thrombin contained in fibrin glues led to maximum hypersensitivity reactions due to the production of anti-bovine Factor V antibodies, which led to its being withdrawn from the French market and replaced by human thrombin (89-93). Aprotinin is a highly immunogenic protein containing 58 amino acids and has an inhibitory activity on serine protease and other wound-healing proteases. Since 1960, 125 anaphylactic reactions after intravenous and local application of fibrin glue have been reported (94, 95). However hypersensitivity to aprotinin remains relatively rare: incidence is about 0.5/100,000 applications, but since 90% of incidents are not declared, an incidence of 5/100,000 applications can be hypothesized (9). Anaphylactic reaction starts within 30 min after application of the fibrin glue and symptoms reported vary: skin symptoms, mild fever and cardiorespiratory arrest. 40% are moderate reactions, 50% are life-threatening and 10% are lethal (94). These reactions involve anti-aprotinin IgE and IgG (antibodies remain in 80% of patients 3.5 months after exposure and persist up to one year) with high histamine rates (96). Generally, repeated use of fibrin sealants containing aprotinin should be avoided.

A case of hypersensitivity reaction several days after application of Beriplast[®] for the treatment of an aneurysm has been reported, manifested by aphasia and mild right hemiparesis.

Subsequent MRI revealed neurological deterioration, possibly due to inflammatory reaction. Full resolution of the symptoms without neurological scars occurred a few hours after administration of systemic steroids. Nine months later, a lymphocyte stimulation test was carried out on the patient's serum and revealed hypersensitivity to the fibrinogen/aprotinin/F XIII mixture (97).

Tranexamic acid and neurotoxicity

Choosing a fibrin glue without aprotinin (Quixil[®]) decreases the hypersensitivity risk. However, its tranexamic acid content can lead to neurotoxicity (edema, seizures), making Quixil[®] contraindicated in neurosurgery or in surgery at high risk of contact with cerebrospinal fluid or dura mater. Tranexamic acid is a specific plasminogen inhibitor able to induce immunogenic reactions (type IV hypersensitivity) (9). Like aprotinin, tranexamic acid is controversial. A recent study, for instance, finds that tranexamic acid is less effective than aprotinin: it significantly decreases the tensile strength of the clot to 40%, as well as the cross-linking of fibrin polymers; consequently, the life span of the clot is reduced (98).

With respect to anti-fibrinolytic agents, a limited number of studies have been carried out and outcomes should be seen in relative terms: indeed, a previous study observed a greater efficacy of tranexamic acid (compared to aprotinin) concerning early hemostasis (improvement in clot percentage: 70.13 +/- 2.02% for tranexamic acid versus 61.22 +/- 2.21% for aprotinin and 61.28 +/- 2.36% for control, $p < 0.05$) (99).

Other adverse effects

Thromboembolic complications and disseminated intravascular coagulation (DICC) can occur if sealant is unintentionally administered intravascularly. Application of fibrin glue must be discontinued immediately if there are signs of hypersensitivity reactions and symptomatic treatment of anaphylactic shock initiated.

Injection of Tissucol[®] and Beriplast[®] in nasal mucosa is contraindicated because of the risk of anaphylaxis (13). Several cases of air embolism by spraying at high pressure (> 2.5 bar) or by spraying at short distances (< 10 cm) have been reported.

The safety of fibrin glue application in nervous tissue has been assessed: a strong and intensive inflammatory reaction was noticed

seven days after application, however spontaneous healing occurred without any residual scar tissue. In the long term, fibrin sealants do not induce nerve damage in cerebral tissue, unless they contain tranexamic acid (100).

AUTOLOGOUS FIBRIN SEALANT

Vivostat[®] and CryoSeal[®] are available in the USA and are attracting growing interest in Europe (CE label since 2000). Unlike conventional fibrin sealant products, this kind of fibrin glue is not a plasma-derived product - it is a medical device.

Manufacture

The automated system allows 4-6 mL of fibrin to be extracted from 120 mL of whole blood. Cryoprecipitation is the method most often employed for a maximum yield of fibrinogen for fibrin sealant manufacturing (CryoSeal[®] system). In this computer-controlled method, whole blood is centrifuged to harvest a poor-platelet fibrin component. Then the sample undergoes freezing at -70°C followed by thawing at 37°C; a second freeze-thaw cycle is carried out at -70°C/+4°C. Finally, the fibrinogen component is activated by the addition of calcium chloride. This automated system leads to fibrinogen precipitation at a rate of 20 mg/mL in 1 hour (11).

The Vivostat[®] system is another device which produces fibrin glue by chemical methods. The fibrinogen component is cleaved by batroxobin (a snake venom protease) to release an acid-soluble fibrin 1 monomer and FpA (pH = 4.5). Then the solution is mixed with a pH 10 buffer: the resulting neutral solution is free from thrombin and contains calcium. When fibrin 1 and prothrombin solutions are blended, prothrombin is activated into thrombin, which catalyzes the release of fibrin 2 monomer and FpB from fibrin 1 monomer. Thrombin also activates F XIII, which cross-links soluble fibrin 2 monomer to form a stable insoluble fibrin 2 polymer. Vivostat[®] harvests fibrin at a rate of 20 mg/mL within 1 hour (101, 102). This mode of preparation differs from those of Tissucol[®] and Beriplast[®], in which both FpA and FpB are simultaneously cleaved in order to form a stable fibrin clot; Vivostat[®] produces a fibrin clot by modifying the pH of the solution. When the cross-linking kinetics of the different sealants was assessed, the kinetics of Vivostat[®] was 6- to 20-fold higher than those of Tissucol[®] and Beriplast[®]. Thus, a fibrin clot is formed almost instantaneously with the Vivostat[®] system.

However, the manufacturing techniques used by Vivostat[®] and CryoSeal[®] lack reproducibility with regard to mechanical properties and clinical outcomes. The fibrinogen synthesis rate varies according to numerous factors such as age, sex, alcohol use, BMI, tobacco use (102, 103).

Autologous fibrin glue can be produced in another way that is faster (30 min) and cheaper (10 dollars/10 mL of plasma): from pooled plasma (at least eight donors), precipitating fibrinogen by protamine sulfate. This manufacturing method can yield 100% of the fibrinogen contained in the plasma. The fibrinogen thus harvested has been shown to be entirely clottable, and anti-fibrinolytic agents did not affect the tensile strength and adhesive strength of the fibrin clot. The tensile strength of this fibrin glue was found to be similar to that of Tisseel[®] and adhesive strength was higher (because Tisseel[®] no longer contains F XIII after viral inactivation). The efficiency of this kind of glue is therefore fully satisfactory. A comparative study of protamine-fibrinogen concentrate versus Tisseel[®] showed an improvement in blood loss and duration of bleeding using the former ($V < 0.05$) (103).

Characteristics

After manufacturing, fibrin sealant obtained with the Vivostat[®] system contains low amounts of fibrinogen, albumin, F XIII and tranexamic acid, but does not contain fibronectin or other clottable plasma protein, which is why late hemostasis and tensile strength are poorer compared with other fibrin sealants (104). Since the tranexamic acid-containing formulation of autologous sealant is 10 mM as opposed to 605 mM in Quixil[®], any risk of neurotoxicity should be avoided; however, there is no randomized neurosurgery assay to prove Vivostat[®] safe. In addition, this tranexamic acid concentration appears not to be high enough to

prevent premature clot lysis. Indeed, in vivo, fibrin clots obtained with both Vivostat[®] and CryoSeal[®] systems were partially liquefied after 24 hours and totally liquefied after 2 days. With Tissucol[®], fibrin clots were partially liquefied at day 5 and totally liquefied at day 6. To sum up, autologous fibrin glues offer lower fibrinogen and fibrin concentrations, less cross-linking of fibrin fibers, major batch-to-batch variability and poorer resistance to clot lysis compared with Tissucol[®] (104, 105).

Although fear of virus or prion transmission was the driver for the development of autologous fibrin sealants, it is important to note that bacterial transmission is not diminished with this kind of product (104). Autologous glues are still preferred, despite clinical contraindications or the orthopedic context (postoperative osteomyelitis if fibrin glue contaminated with *S. epidermidis* from patient is used). Several randomized assays were carried out on Vivostat[®], but few were entirely independent of the manufacturers (106-114). A study on prolonged pulmonary air leaks highlighted the efficacy of Vivostat[®], which decreased prolonged air leaks and the duration of chest tube drainage after lung volume reduction. Indeed, postoperative severity scores were significantly lower for patients treated with Vivostat[®] ($p < 0.001$).

Only one comparative study focused on the features (Table V) and efficacy of Vivostat[®], CryoSeal[®] and Tissucol[®] (101). As fibrinogen concentration in the clot is directly linked to its tensile strength, the Vivostat[®] system appears to produce fibrin glue more efficiently than the CryoSeal[®] system, but this remains unproved. However, the quality of the fibrin clot formed via the Vivostat[®] system remains inferior to that of Tissucol[®].

Table 5. Autologous fibrin glues versus fibrin glues from pooled plasma

		CRYOSEAL [®]	VIVOSTAT [®]	TISSUCOL [®]
Fibrinogen component	ALBUMIN	+++	++	++
	FIBRONECTIN	+	-	+
	FIBRINOGEN	++	+++	+++
Thrombin component	ALBUMIN	+++	-	+++
	THROMBIN	+	-	+
Fibrin concentration in the clot (mg/mL)		9.94	16.35	45
Anti-fibrinolytic agent		-	With or without tranexamic acid	Aprotinin

Automatized production of autologous fibrin glue is rapid (30-45 min) and economic (7 euros for 30 mg/2 mL of fibrinogen). Initial investment is nearly 25,000 euros, but the cost is offset by the treatment of numerous massive hemorrhages. Moreover, the high price of fibrin glues from pooled plasma makes autologous fibrin sealants an attractive alternative; especially if large volumes are required (automatized systems are designed to prepare volumes greater than 100mL); nevertheless, quality and reproducibility of the method are not well controlled.

CONCLUSION

Fibrin glues have proved their efficacy in hemostasis, as they mimic the last step of blood clotting. Since the qualitative and quantitative composition of commercially available products differs, their efficiency (tensile strength, elasticity, release kinetics, required volume) differs with the type of fibrin sealant. Moreover, studies show the major role played by F XIII in these sealants' efficacy. To achieve optimum results, each individual situation needs to be evaluated to identify the most suitable glue for each individual patient and surgery.

Although the indications for fibrin glue are currently limited to enhancing hemostasis in surgery, assays emphasize its value as a drug delivery system (for growth factors, stem cells and medicines), improving drug action in the target area. The findings of several studies comparing fibrin glue containing a drug versus the drug alone support this use of fibrin sealant. As for platelet-rich fibrins, their usefulness in tissue engineering is well established and widely reported (especially in chondrogenesis).

However, the presence of anti-fibrinolytic agents in most fibrin glues is an issue of concern, resulting in no improvement of fibrin clot quality, risk of hypersensitivity reactions (aprotinin) or neurological toxicity (tranexamic acid). On this issue, there are few studies involving fibrin glues (especially comparative studies) and some assays can be suspected of commercial bias. Consequently, further studies based on reliable data would be desirable.

Looking ahead, autologous fibrin sealants appear to present advantages compared with fibrin glues from pooled plasma, despite their slightly lower efficiency. Most notably, the risk of viral transmission is removed, since the receiver is his own blood donor. In addition, tolerance of autologous fibrin glues is better and they can be

produced in larger quantities. However, data on autologous glues are currently insufficient, and new studies need to be undertaken.

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