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LOCAL HEMOSTATIC BLOOD PRODUCTS IN HEMOPHILIA CARE: FIBRIN SEALANT AND PLATELET GEL

Second Edition

Thierry Burnouf
Miryana Radosevich
Human Plasma Product Services (HPPS)
Lille, France

Hadi Alphonse Goubran
Cairo University
Cairo, Egypt

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World Federation of Hemophilia
1425 René Lévesque Boulevard West, Suite 1010
Montréal, Québec H3G 1T7
CANADA
Tel. : (514) 875-7944
Fax : (514) 875-8916
E-mail: wfh@wfh.org
Internet: www.wfh.org

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Local Hemostatic Blood Products in Hemophilia Care: Fibrin Sealant and Platelet Gel

Thierry Burnouf, Miryana Radosevich, and Hadi Alphonse Goubran

Introduction

Treatment of bleeding in patients with hemophilia A or B, von Willebrand disease or other coagulation factor deficiencies (e.g., factor XI or factor VII) is based on substitutive therapy by intravenous infusion of plasma-derived factor concentrates or, when available, recombinant coagulation factor products. Another category of hemostatic products produced from human blood/plasma are proving to be helpful topical agents to stop or control bleeding in these patients (including those who have developed an inhibitor), at least in some surgical situations. Fibrin sealant, also called fibrin glue, may reduce or eliminate the need to infuse coagulation factor concentrates during some surgical procedures [1]. Platelet gel, a newer product still much less used in patients with bleeding disorders, may also present clinical benefits. This monograph describes the manufacturing methods and characteristics of these hemostatic products and the clinical indications where they may be used for patients suffering from bleeding disorders.

Fibrin Sealant

Definition and properties

Fibrin sealants are prepared in clinical practice by mixing, at the time of use, two plasma-derived protein fractions: a fibrinogen-rich concentrate and a thrombin concentrate. Mixing fibrinogen and thrombin mimics the last step of the blood coagulation cascade, resulting in the formation of a semi-rigid to rigid fibrin clot that consolidates and adheres to the application site and acts as a fluid-tight sealing agent able to stop bleeding and hold tissues and materials in a desired configuration. Fibrin sealants have hemostatic, sealing, and healing properties [2-5]. Their advantages over synthetic surgical glues include biocompatibility, biodegradability, and the absence of induction of inflammatory reactions or tissue necrosis. Readsorption of the fibrin clot is achieved within days to weeks following application, depending upon the type of surgery, the amount of product used, the density and characteristics of the fibrin clot, and the proteolytic activity of the site treated.

Mode of preparation

The components of fibrin sealants can be prepared from large pools of plasma or from single plasma donations, often described as industrial or "commercial," and blood-bank products, respectively.

Industrial fibrin sealant

Production

For commercial fibrin sealant, the fibrinogen, and now also the thrombin concentrates, are made by industrial fractionation of batches of hundreds or thousands of litres of plasma. Fibrinogen concentrate is usually obtained by precipitation methods to isolate the cryoprecipitate or the Cohn fraction I, from which fibrinogen is further purified [6]. Thrombin is usually obtained by a manufacturing process that includes activation of a pre-purified human prothrombin fraction (similar to the prothrombin complex concentrate) into thrombin, followed by chromatographic purification. Fibrinogen concentrate has high protein content (typically more than 80g/L) and may, depending upon the mode of production, also contain fibronectin, von Willebrand factor, and factor XIII [2]. It may be reconstituted using an antifibrinolytic agent. The concentration of the thrombin concentrate is typically over 500 IU/mL, although preparations with lower potency are used when slower polymerization of the sealant is needed to provide time for tissue adjustment. Both components are usually freeze-dried, but frozen formulations are also available. Thrombin concentrate is solubilized in a calcium chloride solution. Upon mixing of the two components, a strong adhesive fibrin clot is formed either

instantaneously or within a few seconds, depending on the concentration of thrombin and the quality of the fibrinogen preparation.

Viral safety
As industrial products are made from large pools of plasma, measures are taken to ensure an optimal margin of viral safety. As for any plasma-derived products, safety precautions include careful selection of plasma/blood donors; immunological testing of individual plasma donations for human immunodeficiency (HIV) and hepatitis C (HCV) viruses; hepatitis B (HBV) antigen assays; and screening of plasma pools by nucleic acid tests (NAT) against a range of viruses, including HCV, HIV, HBV, and more recently, non-enveloped viruses such as parvovirus B19 (human erythrovirus B19, B19V) and hepatitis A (HAV). Any donations found to be positive for a viral marker are eliminated to control and limit the potential viral load in the manufacturing plasma pool. In addition, both the fibrinogen and thrombin fractions are subjected to one or several robust viral inactivation steps [7] such as solvent-detergent [6], pasteurization [8], vapour-heat treatment, or nanofiltration [9]. Under U.S. and E.U. regulations, these steps must be carefully characterized to demonstrate their ability to reduce the risk from at least the lipid-enveloped viruses.

Current viral inactivation treatments are indeed very efficient against lipid-enveloped viruses, but some are less effective against non-enveloped viruses. The safety of commercial fibrin sealant is thought to be high, but several cases of possible transmission of B19V by one commercial product appear to have been identified in Japan [10-12]. Implementation of NAT for B19V in the starting plasma may, however, now reduce the risks.

Aprotinin
Most of the current commercial preparations are formulated with aprotinin, an antifibrinolytic agent that is used to solubilize the fibrinogen fraction and is expected to slow the degradation of the fibrin clot by the proteolytic enzymes (e.g. plasmin) in body fluids. Newer products tend not to contain aprotinin, however, in part due to its bovine origin and in part due to the fact that its use, which is sometimes debated, needs to be justified by controlled pre-clinical and clinical trials. Recent animal experiments in trauma surgery actually demonstrated that, at least in such applications, aprotinin made no contribution to the immediate or long-term hemostatic performance of two industrial fibrin sealants [13]. Tranexamic acid has been shown experimentally to be a good substitute for aprotinin [14], and is used in at least one commercial product, but it carries the risk of potentially fatal neurotoxicity when used in contact with the central nervous system [15].

Blood bank fibrin sealant

Production
Fibrin sealants can also be prepared from single plasma units processed directly at general or hospital blood banks. In general clinical practice, the plasma donations used as starting material can be obtained from the patient (autologous use) or from another plasma donor (homologous use). Autologous use has the advantage of reducing the risk of transfusion-transmitted disease to that associated with errors in the preparation and administration of the sealant but, for obvious reasons, it is not feasible for patients with bleeding episodes.

Various methods of production of the fibrinogen fraction, usually based on precipitation from whole plasma, have been developed [16-20]. Human cryoprecipitate is, in practice, the usual source of fibrinogen. It is obtained by thawing a freshly frozen plasma unit at close to 2°C to generate the cryoprecipitate. Most of the plasma supernatant is removed, usually by centrifugation. The resulting cryoprecipitate is solubilized at room temperature with residual cryo-poor plasma, recovered aseptically into a syringe, and generally used fresh (or frozen until use). The process typically yields 5-10 mL of fibrinogen solution from 200 mL of plasma. Some blood banks can also prepare the fibrinogen using ethanol, ammonium sulfate, and polyethylene glycol [16, 18, 19, 21]. Cryoprecipitation and ammonium sulfate precipitation appear to provide the highest fibrinogen yield, whereas ammonium sulfate precipitation may allow production of a fibrin sealant with higher tensile strength [22].

In most current situations, the thrombin used for blood bank fibrin sealants comes from a bovine source. Bovine thrombin carries risks of inducing immunological reactions (formation of

cross reactive anti-factor V or anti-thrombin antibodies) and has also raised concerns over transmission of bovine infectious agents, such as bovine spongiform encephalopathy [23]. However, devices to prepare thrombin from single human plasma donations are being made available, raising the prospect of 100% human-derived single-donor fibrin sealants. The fibrinogen concentration in these fibrin sealants is typically close to 20g/L and the formation of the fibrin clot, upon mixing with thrombin at a concentration close to 50 IU/mL, typically takes 2-10 seconds [24, 25]. The strength of the clot, although markedly less than that of industrial fibrin sealants, appears to be enough to fit many clinical applications.

Recent experimental studies have shown that the addition of growth hormone may exert a synergistic effect in augmenting the healing properties of fibrin sealant in anastomosis [26].

Viral safety

Blood bank fibrin sealants made from homologous plasma donations carry a possible risk of transfusion-transmitted diseases. Infection risks are, however, limited by the fact that these products are not made from a pool of plasma donations. At this stage, safety therefore relies on proper donor selection and viral screening of the donations. With the standards of selection and testing currently in place in developed countries, the risk of viral infection by pathogens that are recognized and tested for in single plasma donations is very low. However, emerging infections such as West Nile virus and severe acute respiratory syndrome (SARS) confirm that constant vigilance (for both pooled products and single-donor products) should be in place.

Unlike industrial fibrin sealant, blood bank fibrin sealant is usually not subjected to viral inactivation processing and thus carries a potentially higher risk of transmitting pathogens that are not screened for. However, it is conceivable that the development of pathogen reduction methods applicable to single plasma donations or minipools of plasma [27-30] will make the production of fibrin sealants from viral-inactivated plasma donations possible. Acceptable recovery in clottable fibrinogen (and eventually thrombin) during such viral reduction treatment should nevertheless be obtained, a feature that is not currently guaranteed by most methods [31-35]. Viral reduction methods of single-donor plasma units already licensed in some countries or described in the literature include methylene blue/illumination [36], psoralen/UVA treatment [37], riboflavin/UV [29], nanofiltration [38], and gamma-irradiation [39]. However, some of these methods induce a 20-30% loss of clottable fibrinogen.

Fibrinogen derived from minipools of solvent detergent-treated cryoprecipitate has also been advocated and can enhance the safety of fibrin sealants [40].

There is to date very limited information on the possibility of applying these techniques to the production of single-donor fibrin sealant components.

Human versus bovine thrombin

As clinical interest increases, attention has also focused on the potential risks associated with the use of bovine thrombin as fibrin and platelet activator in both single-donor fibrin sealant and platelet gel [41]. Three risks have been identified. One is the development of anti-bovine factor V antibodies during the first use of the product, that may cross-react with the patient's own factor V and lead, upon reuse of the product, to potentially severe bleeding episodes [42-46] (although other causes for anti-factor V inhibitors have also been identified [45]). The second is the theoretical risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) [47] or other zoonotic agents of bovine origin. Finally, human thrombin preparations were generally found to be of higher purity than bovine products, and thus induce less severe side effects when infused experimentally [48]. Therefore, the use of single-donor human thrombin, rather than a product of bovine origin, appears to be a logical trend for the future clinical use of blood bank fibrin sealant and platelet gel.

Platelet Gel

Definition and properties

Platelet gel may be of interest in hemophilia care, but it is still of limited clinical use in these patients. This newly introduced biomaterial is

obtained by combining a platelet-rich blood fraction, such as a platelet concentrate or platelet-rich plasma, with calcified thrombin [49-51]. This produces the same physiological reaction as fibrin sealant and results in the formation of a soft, gel-like biomaterial. In addition, activation of platelets by thrombin leads to the release of several growth factors from the platelet granules [51].

Mode of preparation

In contrast to fibrin sealant, platelet gel is exclusively produced using single-donor, homologous or autologous platelet concentrates, and not from pooled platelets. The platelet concentrate can be obtained from a blood bank, and is produced by standard whole blood centrifugation or by platelet apheresis [52]. For patients not suffering from bleeding disorders, a small amount of their own blood (50 mL) may be collected prior to clinical intervention and processed using special devices to isolate a platelet-rich fraction. Platelet fractions are then mixed, prior to clinical application, with thrombin. The mixture results, within 5-20 seconds, in the formation of a fibrin-enriched, "gelatin-like" substance that contains platelet-derived cell growth-promoting factors [51]. The therapeutic use of autologous platelet-leukocyte gel is a relatively new approach similar to platelet gel, which might stimulate and accelerate soft-tissue and bone healing thanks to the release of platelet growth factors [53].

Since platelet concentrates contain less fibrinogen than the fibrinogen concentrate used in fibrin sealant, the strength of the resulting gel is significantly lower and the product cannot be used as efficiently to stop bleeding. The platelet-rich blood fraction can possibly be pre-mixed with cryoprecipitate which, upon mixing with thrombin, produces a product often referred to as "platelet glue" or "platelet-cryo glue" with better adhesive properties and higher resistance to body fluids and pressure than platelet gel [54].

Safety

As for single-donor homologous fibrin sealant, the viral safety of homologous platelet gel relies upon appropriate donor selection and screening of platelet concentrates. Viral inactivation methods applicable to single-donor platelet concentrate, using psoralen/UVA [30] or riboflavin/illumination [55], are also being developed and could be considered for the production of platelet gel as long as the release of growth factors and the capacity to form the fibrin gel are not altered upon treatment.

For the reasons discussed earlier, the use of single-donor human thrombin also appears to be a future trend for this type of biomaterial [41]. Single-donor human thrombin obtained by activation of plasma has been found to induce the release of platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) from human platelets in concentrations at least as high as those measured when using bovine thrombin [24].

Physiological properties

Thrombin-induced activation of platelets releases PDGF, TGF-β, epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF), which are believed to be sequestered and concentrated in the gel. PDGF plays a role in periodontal regeneration, and TGF-β has a very potent effect on cells associated with bone structure [56]. Due to its fibrin-rich gel composition and the presence of these growth factors, platelet gel allows for the moulding of graft material, ensures secure placement in tissue defects, and promotes cell migration, vascular invasion, and wound healing [56-59].

Platelet gels are currently used increasingly in oral and maxillofacial surgery [50], as well as in orthopedic and reconstructive surgery [49]. It is possible that, in combination with fibrin sealants, they could be of special benefit to hemophilia patients. However, the clinical effects of platelet gels remain poorly documented. Controlled clinical studies are needed to provide objective evidence of the claimed clinical benefits. Since both platelet and leukocyte counts may influence the growth factor content [60], as well as the thrombin concentration and stability, standardized production methods of platelet concentrates should be encouraged. The concentration of major platelet growth factors applied to tissues should be determined and standardized to ensure reproducibility in the clinical outcome.

Mode of Application of Fibrin Sealants and Platelet Gels

The two components of fibrin sealants (or platelet gels) can be applied sequentially or simultaneously using a dual-syringe system that dispenses the two components (similar to the dispensing of epoxy glues used for household repair) [61]. Fibrinogen and thrombin are ejected from their respective channels by thumb pressure and mixed externally at the tip of the cannula or in a mixing chamber within a dual cannula delivery head. Longer single or dual Teflon tubes are used for endoscopic delivery via upper gastrointestinal endoscopes (in cases of bleeding esophageal varices [62]), bronchoscopes or laparoscopic devices. In the case of fibrin sealant, local application produces a fibrin fibre network, whereas spraying allows the formation of a dense fibrin film that may be helpful for hemostasis of larger bleeding surfaces. A separate compressor connection is needed to spray the components of fibrin sealant, using a multilumen head on the desired surface. A simpler dual channel pump sprayer (similar to perfume dispensers) has also been proposed, as well as a gas-propeller. The various applicators are presented in a comprehensive review paper [61].

The blood-derived biomaterial may also be pre-mixed (e.g. with antibiotics, bone chips) for subsequent sealant application in cavities. Platelet gel is often pre-mixed with bone graft or moulding material prior to clinical application. Adhesive products comprised of alternate layers of collagen and fibrinogen freeze-dried together as a composite are also available in some countries for use as a surgical dressing. Other areas of innovation include expandable foams and spray powders that may allow people with hemophilia to rapidly control traumatic hemorrhages prior to hospital treatment [5]. The potential use of fibrin sealant in a bandage form for trauma patients has also been considered [3].

Clinical Use of Fibrin Sealants

Fibrin sealant has been used in patients suffering from hemophilia A and B, von Willebrand disease, factor XI [5, 63, 64] and other coagulation factor deficiencies [1, 65]. In hemophilia patients, fibrin sealants have been used extensively and successfully to carry out dental extractions, to perform orthopedic and non-orthopedic surgeries, and for circumcision. This has been shown to reduce the need for intravenous substitutive therapy [66]. However, there are still relatively few well-controlled studies of the clinical efficacy of fibrin sealants in patients with bleeding disorders [67], in part due to the difficulty of defining valid and measurable clinical endpoints. In patients without bleeding disorders, endpoints may include improvement in hemostasis when using fibrin sealants compared to a placebo or a procedure that's considered a "standard of care." In patients suffering from bleeding disorders, a reduction in the need for substitution therapy with factor concentrate may be considered as a valid clinical outcome [3]. Both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) have issued guidance documents regarding clinical trials of fibrin sealants [68, 69].

Dental extraction and oral surgery

Fibrin sealant is used to achieve local hemostasis following dental extraction in hemophilia patients. The use of fibrin sealant in 118 tooth extractions in patients with hemophilia A or B or severe von Willebrand disease demonstrates the benefits regarding reduction of blood loss [70, 71]. Requirements for systemic replacement therapy and severity of secondary hemorrhage appeared to diminish. Optimization of the method of application, however, remains to be defined [70, 71].

Locally prepared fibrin sealants have now been widely used and have proven useful for dental surgery in Thailand. Good local hemostatic, adhesive, and sealant effects were observed in 43 patients with bleeding disorders, including hemophilia. Only 3 cases (7%) required blood components compared to all 50 in the control group, who did not receive fibrin sealants (100%). Fibrin sealant was found to minimize blood product consumption, decrease medical workload, reduce medical cost, and increase patient convenience and satisfaction [72]. The value of fibrin sealant appears enhanced in children with coagulation factor inhibitors. It was shown that the use of fibrin sealant is less expensive than substitutive therapy, and especially valuable in the treatment of children

with hemophilia A with antibodies against factor VIII [73].

Combining fibrin sealants with tranexamic acid mouthwash helps reduce the high fibrinolytic activity in saliva and improve the outcome in severe patients. In a study of 80 patients with various types of bleeding disorders undergoing 135 extractions without preventive replacement therapy, local hemostasis was achieved using fibrin sealants alone. Secondary bleeding occurred in 9 of 12 patients with severe hemophilia, but when swish-and-swallow rinses of tranexamic acid were used before and after the dental extractions and the concentration of antifibrinolytic aprotinin was increased, only 3 of 25 hemophilia patients suffered from secondary bleeding [74].

Fibrin sealant also proved beneficial in repairing bone defects adjacent to titanium dental implants [75].

Although larger randomized controlled studies of fibrin sealant preparations in dental surgery in hemophilia patients are needed, existing data support its use as a good hemostatic tool.

Orthopedic operations

Fibrin sealant is recognized to be an excellent tool in orthopedic and trauma surgery [76, 77]. The positive effect of some fibrin sealants in wound healing has been conclusively demonstrated, and they have also been shown to exhibit osteoconductive properties. Fibrin sealants may be applied in combination with implantation material (tricalcium phosphate plus bone and gelatin, as well as demineralized bone matrix) to facilitate application and control of bony morphology [78]. Fibrin sealant not only facilitates hemostasis, it permits tissue fixation, enhances plasticity of granular implant material, and stimulates fibroblast growth. Clinical results are especially convincing in hemophilia patients.

In a consecutive series of 16 patients with hemophilia, 21 total knee replacements were carried out for hemophilic arthropathy under factor VIII replacement therapy via continuous infusion and using fibrin glue to facilitate hemostasis. The follow-up evaluation undertaken between 2 and 10 years after the operation (mean 5.6 years) showed satisfactory knee scores and a long-lasting improvement in quality of life [79]. Fibrin sealant has also been used in joint correction [72].

Circumcision

Social and cultural integration of boys with hemophilia is one of the most important cornerstones of modern hemophilia therapy. Circumcision is an important ritual for Muslims and Jews and an important social problem for the hemophilia patient and his family [80]. Circumcision can be fatal in hemophilia patients unless performed under cover of the missing coagulation factor.

In a study of 11 patients with hemophilia (10 with hemophilia A, and 1 with hemophilia B, with age range 6-14 years), circumcision was carried out using fibrin glue for local hemostasis to reduce the duration and intensity of clotting factor replacement. None of the patients had significant bleeding or complications. The total costs were significantly reduced when compared to patients receiving only clotting factor replacement. Fibrin sealant limited the need for factor substitution after circumcision and reduced the high cost of treatment [81]. Fibrin sealant is now widely used during circumcision of hemophilia patients and several studies have confirmed that its use is effective, safe, and cheaper than infusion of factor concentrate [70]. In most cases, the need to perform systemic substitutive therapy is avoided and, when required, lower doses of factor VIII or factor IX products need to be infused. Fibrin sealant has also been used in various urological applications [82].

Traumatology

Use of fibrin sealant has been described in traumatology to seal diffuse bleeding in the muscles or localized hematomas, in combination with sufficient substitution of coagulation factor carried out right after trauma and continued during wound healing and mobilization [83]. Fibrin sealant proved to be a remarkable tool in sealing and promoting healing in intra-abdominal traumas and liver resections [84] and hepatobiliary surgeries [85]. It has also been used successfully in burns [86], but the real superiority of fibrin sealant over conventional sutures is seen in traumas involving peripheral nerves and nerve trunks [87], as well as in plastic reconstruction [25, 54].

Conclusion

Fibrin sealant products, both commercial and made locally by blood banks, have been proven safe and beneficial in hemophilia care, particularly in dental surgery, orthopedic surgery, and circumcision. They provide life-saving control of hemorrhage, reduce coagulation factor supplementation, and allow some reduction in medical cost. One might expect that the role of fibrin glues, and possibly platelet gels, will continue to expand to new areas in the hemophilia therapeutic field.

Special attention should be devoted to the application techniques, including the devices used for these products, as they may greatly influence the clinical outcome. Further controlled clinical studies should be designed to establish the clinical benefits of platelet gel, especially in relation to the concentration of platelet growth factors present in the biomaterial.

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1425 René Lévesque Blvd. W., Suite 1010 Montréal, Québec H3G 1T7 CANADA
Tel.: +1 (514) 875-7944 Fax: +1 (514) 875-8916
www.wfh.org