

Help for the Haemostasis Market

Jaap Koopman and Jan Ohrstrom at Profibrix examine how haemostats based on fibrin are able to harness the natural blood clotting system to treat and prevent excessive bleeding

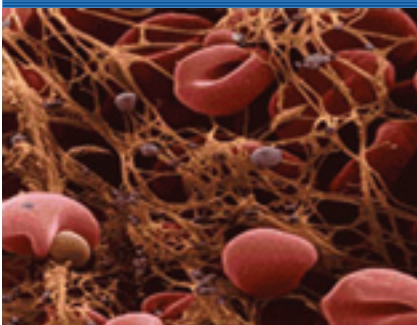
BLEEDING IS AN UNMET MEDICAL NEED

In developed countries, over 200,000 people bleed to death every year, and in developing countries, this number is estimated to be over 10 times higher. In the majority of these cases, bleeding is the result of traumatic injuries and people often die before or during transportation to appropriate hospital care. Additionally, approximately five million people worldwide are hospitalised because of severe bleeding and even within a hospital setting, severe bleeding often presents a challenging situation to the emergency medical professionals.

Apart from traumatic injuries, uncontrolled bleeding that requires medical intervention also occurs in a variety of surgical settings and is a factor that can negatively affect the outcome of surgery. One of the challenges in treating bleeding is that it occurs in many different forms, ranging from diffuse, to oozing from soft tissues like the liver and spleen, to high pressure bleeding from arterial injuries. The extent of haemorrhage is a function of the degree of vascular disruption, blood pressure at the site of injury, the clotting potential of the blood of the patient and the time from injury to treatment.

The reason why bleeding, even in a moderate form, can present itself as a medical need is mainly due to the fact that the natural clotting system is slow to respond to injuries that result in blood loss. This 'delayed' response is a consequence of the many innate or natural control mechanisms in place to prevent the occurrence of excessive blood clotting, which is the opposite of bleeding, and can lead to obstruction of blood flow and result in heart attacks, strokes and other thrombotic events. On top of that, the structural elements in the blood that eventually form the blood clot are only available in a limited amount, and are consumed rapidly during bleeding. This consumption of structural clotting factors, together with dilution by the administration of resuscitation fluids, can reduce the clotting potential of the blood, resulting in serious coagulopathy, whereby even the smallest bleeds (such as bleeding through suture holes) cannot be stopped without intervention.

Figure 1: The haemostatic plug is made up of fibrin strands that act as a scaffold for red and white blood cells and platelets



NATURAL BLOOD CLOTTING

Natural blood clotting is initiated as a result of blood vessel wall injury which leads to exposure of tissue factor, triggering a complex cascade of enzymatic reactions and cellular

interactions that ultimately lead to the formation of the haemostatic plug that seals and repairs the injury. The major structural element of the haemostatic plug is fibrin, a protein polymer that forms the scaffold for the cellular elements, platelets and other blood cells that, together with the fibrin fibres, comprise the haemostatic plug (see Figure 1).

An important part of the regulation of blood clotting takes place at the level of fibrin formation and is described in the 'haemostatic balance theory' first formulated by Tage Astrup in 1958 (1). The haemostatic balance describes the dynamic equilibrium between the reactions that lead to fibrin formation (coagulation) and those that lead to the breakdown of fibrin into soluble degradation products (fibrinolysis). Disruption of this equilibrium can lead to a thrombotic event (excessive coagulation/insufficient fibrinolysis) or bleeding event (insufficient coagulation/excessive fibrinolysis). The central protein in this haemostatic balance is the insoluble fibrin polymer, which is formed from its soluble precursor: fibrinogen.

FIBRINOGEN AND FIBRIN

Fibrinogen, also known as Factor I, is one of the largest blood proteins identified to date. It is synthesised in the liver and is present in blood at levels of one to two grams per litre in healthy individuals. The conversion of soluble fibrinogen into insoluble fibrin polymers is triggered by the active enzyme thrombin. Thrombin is generated from its inactive precursor prothrombin (Factor II) at the end of the enzymatic coagulation cascade that is initiated by tissue factor. Generation of thrombin is of paramount importance for natural blood clotting and therefore it is regulated at many different levels within the coagulation cascade. Several excellent reviews addressing thrombin generation have been published recently (2,3).

Both fibrinogen precursor and the fibrin polymer have extraordinary characteristics (4-6). The fibrinogen molecule is made up of three domains, one central domain and two distal domains that are connected to the central domain by a coiled region of 112 amino acids. The molecule is approximately 45nm in length and the coiled region is interrupted by a short non-helical stretch that is particularly susceptible to proteolytic degradation (7).

After activation by thrombin, newly exposed polymerisation sites in the central domain interact with the distal domains of other activated fibrinogen molecules and form staggered, overlapping, two stranded fibrin polymers (see Figure 2, page 34).

Measurements of the mechanical properties of fibrin polymers have demonstrated that they are among the strongest fibres in nature and they show extreme elasticity (8). These properties

make fibrin the ideal scaffold for the haemostatic plug that must be stiff to stop haemorrhage, but with enough elasticity to resist shear forces in the blood without breaking. The proteolytic sensitive domains in the fibrinogen sub-units ensure timely degradation of the fibrin polymer to prevent obstruction of the blood flow after tissue repair has been completed.

Enhancement of the natural blood clotting system by increasing the availability of fibrinogen and thrombin – the two critical components for fibrin formation – presents excellent opportunities to develop therapeutics and products to treat and prevent severe blood loss.

HAEMOSTASIS PRODUCTS THAT HARNESS NATURAL BLOOD CLOTTING

Haemostasis products can be divided into two categories:

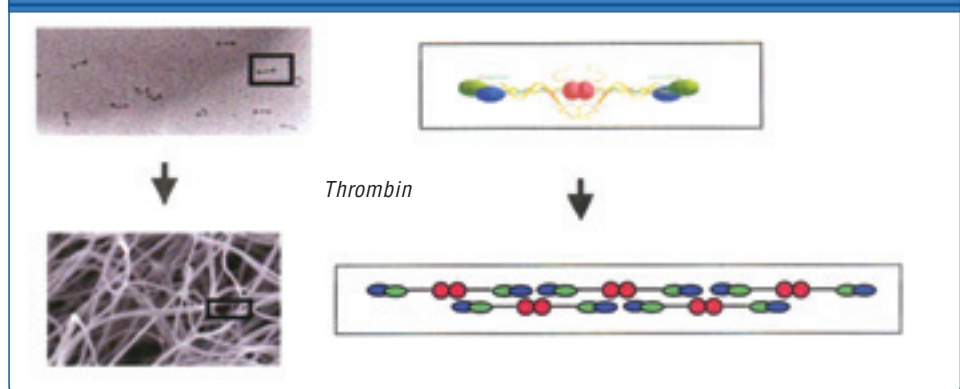
1. Topical products to treat active compressible, localised bleeding
2. Infusible products to treat non-compressible, internal bleeding or prevent bleeding

Topical Haemostats

The 'ideal' topical haemostat should have the following characteristics:

- Safety – The haemostatic agent should be as safe as possible for the patient. The product should not induce overt clotting systemically and therefore must pass rigorous safety tests in accordance with the EMEA/FDA guidelines for the industry.
- Ease of use and readily available – Bleeding is often an unexpected event and requires immediate treatment.

Figure 2: Fibrin formation is a two step process: thrombin activates the fibrinogen precursor molecule; it subsequently polymerises into fibrin strands



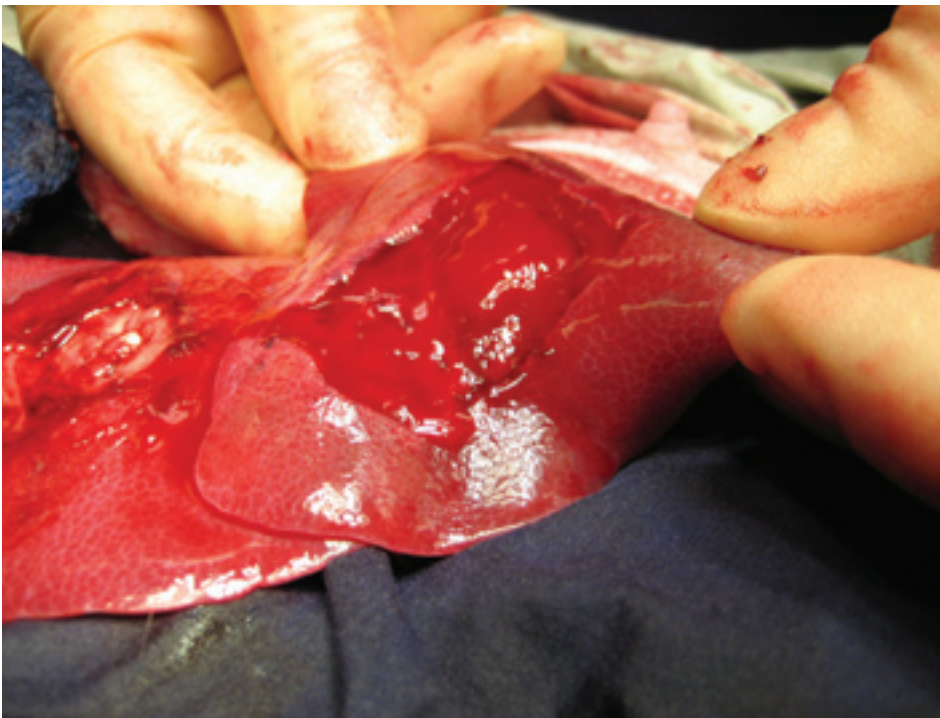
Haemostatic products should therefore be easy to use, available and stable under a variety of conditions and temperatures.

- Efficacious on many different types of injuries – Injuries can vary from a large surface area with oozing bleeding to deep penetrating wounds with high pressure bleeds. Haemostatic products should therefore have the flexibility to treat a range of different bleeds.
- Biodegradable – A haemostatic agent that is biodegradable allows the surgeon the most flexibility in controlling bleeding during the surgical procedure by allowing the agent to remain in place to prevent re-bleeding.
- Cost – The product price should reflect the performance of the product and be at a level where it allows the surgeon to choose the right product in the appropriate situation

Over the years, a wide variety of products have been developed to help control surgical bleeding that cannot be managed by standard surgical practices, such as ligation, cautery or sutures (8). The topical products can be sub-divided according to their mechanism of action to stop bleeding (see Table 1).

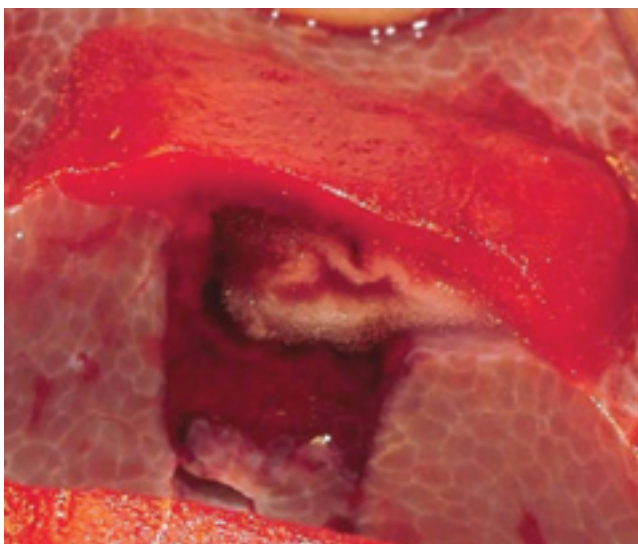
Table 1: A selection of topical haemostasis products currently on the market or in development classified according to mechanism of action

Type of product	Examples	Preparation time (minutes)	Haemostatic potency	Mechanism of action	Flexibility
Mechanical	Gauze, Surgicel, Avitene, Gelfoam/ Surgifoam	<1	Stops mild bleeding	<ul style="list-style-type: none"> ● Pressure/absorption ● Activation endogenous clotting cascade 	<ul style="list-style-type: none"> ● Large surface area; limited endoscopic utility ● Can often be left in place
Active thrombin containing products	Bovine thrombin, Recothrom, Surgiflo, FloSeal, Evithrom	3-5	Stops mild to moderate bleeding	<ul style="list-style-type: none"> ● Accelerated fibrin formation by exogenous thrombin ● Pressure/absorption 	<ul style="list-style-type: none"> ● Mostly used with absorbable collagen/ gelatine sponge ● Endoscopic use limited
Liquid fibrinogen/thrombin containing products	Tisseel, Beriplast, Evicel, Bolheal	5-20	Stops mild to moderate bleeding	<ul style="list-style-type: none"> ● Fibrin formation through exogenous thrombin just haemostasis 	<ul style="list-style-type: none"> ● Endoscopic use ● Utility broader than
Dry fibrinogen/thrombin sheets and pads	Tachosil, Tachocomb, fibrin patch (in development)	<1	Stops moderate to severe bleeding	<ul style="list-style-type: none"> ● Fibrin formation through exogenous thrombin and fibrinogen ● Absorption and pressure 	<ul style="list-style-type: none"> ● Cannot always conform to wound ● No endoscopic use possible
Dry powder fibrinogen/ thrombin product	Fibrocaps (in development)	1-2	Stops moderate to severe bleeding	<ul style="list-style-type: none"> ● Fibrin formation through exogenous thrombin and fibrinogen ● Light pressure 	<ul style="list-style-type: none"> ● Powder conforms to wound ● Endoscopic use possible ● Utility broader than just haemostasis



complete clotting cascade, which makes them very effective and independent of the fibrinogen coagulation status of the patients.

Several formulations of fibrinogen and thrombin have been developed, but the most well known format is the liquid fibrin sealant product, in which the fibrinogen and thrombin are stored separately as frozen liquid or lyophilised powder. Before use, both components need to be reconstituted or thawed and loaded into a two-compartment applicator device that allows mixing of the two components and delivery to the wound. Because of the laborious preparation, the liquid products need thorough instruction of the staff and training of the end-user, issues that can translate into limited use of an otherwise effective formulation.



Formulations in which the fibrinogen and thrombin are provided in a dry form and coated onto a sheet or dressing have been developed, and greatly facilitate the ease of use. A disadvantage of the pre-coated patches or dressings is that they are delivered as a dry, breakable sponge (until wetted), cannot always conform to the shape of the injury and are difficult to use on deep penetrating wounds. Furthermore, the pre-coated products currently available carry a significant cost premium when compared to the liquid fibrin sealants and are not currently available in the US.

A new promising development in the field of fibrin sealants is a novel dry-powder fibrin sealant formulation, where both active components are spray-dried individually, but blended together providing a single, room temperature, stable dry powder blend that can be applied directly to the wound without reconstitution. This manufacturing process results in the creation of soluble micro-particles with free-flowing properties and therefore retains the advantage of conforming easily to the shape of the injury. These unique product characteristics mean that Fibrocaps can be used outside the hospital and provide immediate treatment. Dry-powder fibrin sealant formulation has demonstrated its effectiveness in stopping surgical bleeding in animal models and, unlike currently available liquid fibrin sealants, is stable at ambient temperatures and easy to use. Although the fibrinogen/thrombin products bypass the clotting cascade and provide high levels of thrombin to the wound, they have favourable safety profiles when used properly as per the manufacturer's instructions because the natural thrombin inhibitors like anti-thrombin III, alpha-2-macroglobulin and fibrin itself prevent excessive coagulation.

A large group consists of products that can be classified as mechanical haemostats and do not contain active enzymes or clotting factors. These products work through mechanisms of absorption, pressure, vasoconstriction or activation of the endogenous clotting system. This group is sometimes described as passive haemostats.

A second group of products contains active thrombin, either alone or in combination with one of the mechanic haemostats. These products work by enhancing fibrin formation through locally increasing the amount of active thrombin to a supra physiological level, but they do depend on the endogenous fibrinogen content for efficacy.

The third group of topical haemostats are considered to be the most powerful and contain both active thrombin and fibrinogen clotting proteins (10). These products work by providing excess fibrinogen and thrombin at the site of injury and bypass the

The total market for the topical haemostats, excluding staples, sutures, and so on, is estimated to be around €2 billion (11).

Infusible Haemostats

The most used infusible products to treat or prevent bleeding are platelet concentrates from donor blood, FVIII derived from blood

or produced with recombinant technology and recombinant FVIIa. Other blood derived products such as fresh frozen plasma and the prothrombin complex are also used to treat severe haemorrhage.

In recent years, many scientific reports have demonstrated that fibrinogen is the first clotting factor that drops below a critical level during severe bleeding and that administration of fibrinogen concentrates restores normal blood clotting (12).

Intravenous applications of fibrinogen have gained momentum in Europe and the market leader in this field is the Haemocomplettan P product (CSL Behring, Marburg, Germany). This product is derived from human donor blood and the average dosage of fibrinogen given is 3-5g per treatment. Several new indications for IV fibrinogen have been reported, and a recent study indicated that an increased fibrinogen level could even compensate for a shortage of platelets, suggesting that fibrinogen could be used for the treatment of thrombocytopenia (13).

If these developments continue in the future, the market demand for fibrinogen could be bigger than the plasma fractionation industry can supply and alternative production platforms may be required. Two such developments are ongoing in Leiden, Netherlands, where a transgenic production system in the milk of transgenic cows has been established, as well as a mammalian cell culture system for the production of human fibrinogen. Both

systems will have to demonstrate that the recombinant fibrinogen products are equivalent to plasma derived fibrinogen in structure and function and controlled randomised clinical trials need to be performed to demonstrate that they also work efficiently as infusible haemostats.

CONCLUSION

Haemostasis products based on a combination of fibrinogen and thrombin for topical use or on fibrinogen alone for systemic application, have demonstrated to be capable of stopping or preventing severe bleeding. Promising new developments to improve the ease of use and establish alternative methods of production for fibrinogen are in progress and are expected to deliver new products to the haemostasis market over the coming years.

About the authors



Jaap Koopman, PhD is CEO of ProFibrin BV based in Leiden, Netherlands. In January 2004, he founded ProFibrin and led the company through a seed financing round in January 2005 and the first round of venture funding in February 2007. He has over 20

years' experience in biomedical research, focusing on various aspects of haemostasis, and fibrinogen and fibrin formation in particular. He has published over 30 peer reviewed scientific papers and has received several national and international scientific awards. Jaap is a member of the Council of the International Fibrinogen society and is co-chairman of the ISTH sub-committee for fibrinogen standardisation.

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Jan Ohrstrom, MD is COO and President of ProFibrin Inc, located in Seattle, Washington. Jan has nearly 20 years' biotech industry experience based in the EU and US. He started his industry career at Novo Nordisk A/S in 1990 and held various positions of growing

seniority within Development and Business and Marketing. In 2000, Jan joined the Senior Management Team at ZymoGenetics Inc in Seattle, where he was actively involved in building the company's development and business infrastructure. During his career, he has managed several BLA submissions. Jan joined and incorporated ProFibrin in the US in 2008. He has an MD from University of Copenhagen, where he was trained as a surgeon, and had six years of clinical experience prior to joining the industry. Email: j.ohrstrom@profibrin.com

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