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(54) Title: SOLID DRESSING FOR TREATING WOUNDED TISSUE

(57) Abstract: Disclosed are solid dressings for treated wounded tissue in mammalian patients, such as a human, comprising a haemostatic layer consisting essentially of a fibrinogen component and thrombin, wherein the thrombin is present in an amount between 0.250 Units/mg of fibrinogen component and 0.062 Units/mg of fibrinogen component. Also disclosed are methods for treating wounded tissue.

## SOLID DRESSING FOR TREATING WOUNDED TISSUE

### FIELD OF THE INVENTION

[0001] The present invention relates to a solid dressing for treating wounded tissue in a mammalian patient, such as a human.

### BACKGROUND OF THE INVENTION

[0002] The materials and methods available to stop bleeding in pre-hospital care (gauze dressings, direct pressure, and tourniquets) have, unfortunately, not changed significantly in the past 2000 years. *See* L. Zimmerman *et al.*, *Great Ideas in the History of Surgery* (San Francisco, Calif.: Norman Publishing; 1993), 31. Even in trained hands they are not uniformly effective, and the occurrence of excessive bleeding or fatal hemorrhage from an accessible site is not uncommon. *See* J. M. Rocko *et al.*, *J. Trauma* 22:635 (1982).

[0003] Mortality data from Vietnam indicates that 10% of combat deaths were due to uncontrolled extremity hemorrhage. *See* SAS/STAT Users Guide, 4th ed. (Cary, N.C.: SAS Institute Inc; 1990). Up to one third of the deaths from ex-sanguination during the Vietnam War could have been prevented by the use of effective field hemorrhage control methods. *See* SAS/STAT Users Guide, 4th ed. (Cary, N.C.: SAS Institute Inc; 1990).

[0004] Although civilian trauma mortality statistics do not provide exact numbers for pre-hospital deaths from extremity hemorrhage, case and anecdotal reports indicate similar occurrences. *See* J. M. Rocko *et al.* These data suggest that a substantial increase in survival can be affected by the pre-hospital use of a simple and effective method of hemorrhage control.

[0005] There are now in use a number of newer haemostatic agents that have been developed to overcome the deficiencies of traditional gauze bandages. These haemostatic agents include the following:

- Microporous polysaccharide particles (TraumaDEX®, Medafor Inc., Minneapolis, MN);
- Zeolite (QuikClot®, Z-Medica Corp, Wallington, CT);
- Acetylated poly-N-acetyl glucosamine (Rapid Deployment Hemostat™ (RDH), Marine Polymer Technologies, Danvers, MA);
- Chitosan (HemCon® bandage, HemCon Medical Technologies inc., Portland OR);
- Liquid Fibrin Sealants (Tisseel VH, Baxter, Deerfield, IL)
- Human fibrinogen and thrombin on equine collagen (TachoComb-S, Hafslund Nycomed Pharma, Linz, Austria);
- Microdispersed oxidized cellulose (m•doc™, Alltracel Group, Dublin, Ireland);
- Propyl gallate (Hemostat™, Analytical Control Systems Inc., Fishers, IN);
- Epsilon aminocaproic acid and thrombin (Hemarrest™ patch, Clarion Pharmaceuticals, Inc);
- Purified bovine corium collagen (Avitene® sheets (non-woven web or Avitene Microfibrillar Collagen Hemostat (MCH), Davol, Inc., Cranston, RI);
- Controlled oxidation of regenerated cellulose (Surgicel®, Ethicon Inc., Somerville, NJ);
- Aluminum sulfate with an ethyl cellulose coating (Sorbastace Microcaps, Hemostace, LLC, New Orleans, LA);
- Microporous hydrogel-forming polyacrylamide (BioHemostat, Hemodyne, Inc., Richmond VA); and
- Recombinant activated factor VII (NovoSeven®, NovoNordisk Inc., Princeton, NJ).

These agents have met with varying degrees of success when used in animal models of traumatic injuries and/or in the field.

[0006] One such agent is a starch-based haemostatic agent sold under the trade name TraumaDEX™. This product comprises microporous polysaccharide particles that are poured directly into or onto a wound. The particles appear to exert their haemostatic effect by absorbing water from the blood and plasma in the wound, resulting in the accumulation and concentration of clotting factors and platelets. In two studies of a lethal groin wound model, however, this agent showed no meaningful benefit over standard gauze dressings. *See* McManus *et al.*, Business Briefing: Emergency Medical Review 2005, pp. 76-79 (presently available on-line at [www.touchbriefings.com/pdf/1334/Wedmore.pdf](http://www.touchbriefings.com/pdf/1334/Wedmore.pdf)).

[0007] Another particle-based agent is QuickClot™ powder, a zeolite granular haemostatic agent that is poured directly into or onto a wound. The zeolite particles also appear to exert their haemostatic effect through fluid absorption, which cause the accumulation and concentration of clotting factors and platelets. Although this agent has been used successfully in some animal studies, there remains concern about the exothermic process of fluid absorption by the particles. Some studies have shown this reaction to produce temperatures in excess of 143°C *in vitro* and in excess of 50°C *in vivo*, which is severe enough to cause third-degree burns. *See* McManus *et al.*, Business Briefing: Emergency Medical Review 2005, at 77. The exothermic reaction of QuikClot™ has also been observed to result in gross and histological tissue changes of unknown clinical significance. Acheson *et al.*, *J. Trauma* 59:865-874 (2005).

[0008] Unlike these particle-based agents, the Rapid Deployment Hemostat™ appears to exert its haemostatic effect through red blood cell aggregation, platelet activation, clotting cascade activation and local vasoconstriction. The Rapid Deployment Hemostat™ is an algae-derived

dressing composed of poly-N-acetyl-glucosamine. While the original dressing design was effective in reducing minor bleeding, it was necessary to add gauze backing in order to reduce blood loss in swine models of aortic and liver injury. *See* McManus *et al.*, Business Briefing: Emergency Medical Review 2005, at 78.

[0009] Another poly-N-acetyl-glucosamine-derived dressing is the HemCon™ Chitosan Bandage, which is a freeze-dried chitosan dressing purportedly designed to optimize the mucoadhesive surface density and structural integrity of the chitosan at the site of the wound. The HemCon™ Chitosan Bandage apparently exerts its haemostatic effects primarily through adhesion to the wound, although there is evidence suggesting it may also enhance platelet function and incorporate red blood cells into the clot it forms on the wound. This bandage has shown improved hemostasis and reduced blood loss in several animal models of arterial hemorrhage, but a marked variability was observed between bandages, including the failure of some due to inadequate adherence to the wound. *See* McManus *et al.*, Business Briefing: Emergency Medical Review 2005, at 79.

[0010] Liquid fibrin sealants, such as Tisseel VH, have been used for years as an operating room adjunct for hemorrhage control. *See* J. L. Garza *et al.*, J. Trauma 30:512-513 (1990); H. B. Kram *et al.*, J. Trauma 30:97-101(1990); M. G. Ochsner *et al.*, J. Trauma 30:884-887 (1990); T. L. Matthew *et al.*, Ann. Thorac. Surg. 50:40-44 (1990); H. Jakob *et al.*, J. Vasc. Surg., 1:171-180 (1984). The first mention of tissue glue used for hemostasis dates back to 1909. *See* Current Trends in Surgical Tissue Adhesives: Proceedings of the First International Symposium on Surgical Adhesives, M. J. MacPhee *et al.*, eds. (Lancaster, Pa.: Technomic Publishing Co; 1995). Liquid fibrin sealants are typically composed of fibrinogen and thrombin, but may also contain Factor XIII/XIIIa, either as a by-product of fibrinogen purification or as an added ingredient (in certain applications, it is therefore

not necessary that Factor XIII/Factor XIIIa be present in the fibrin sealant because there is sufficient Factor XIII/XIIIa, or other transaminase, endogenously present to induce fibrin formation). As liquids, however, these fibrin sealants have not proved useful for treating traumatic injuries in the field.

**[0011]** Dry fibrinogen-thrombin dressings having a collagen support (*e.g.* TachoComb™, TachoComb™ H and TachoSil available from Hafslund Nycomed Pharma, Linz, Austria) are also available for operating room use in many European countries. *See* U. Schiele *et al.*, *Clin. Materials* 9:169-177 (1992). While these fibrinogen-thrombin dressings do not require the pre-mixing needed by liquid fibrin sealants, their utility for field applications is limited by a requirement for storage at 4°C and the necessity for pre-wetting with saline solution prior to application to the wound. These dressings are also not effective against high pressure, high volume bleeding. *See* Sondeen *et al.*, *J. Trauma* 54:280-285 (2003).

**[0012]** A dry fibrinogen/thrombin dressing for treating wounded tissue is also available from the American Red Cross (ARC). As disclosed in U.S. Patent No. 6,762,336, this particular dressing is composed of a backing material and a plurality of layers, the outer two of which contain fibrinogen (but no thrombin) while the inner layer contains thrombin and calcium chloride (but no fibrinogen). While this dressing has shown great success in several animal models of hemorrhage, the bandage is fragile, inflexible, and has a tendency to break apart when handled. *See* McManus *et al.*, *Business Briefing: Emergency Medical Review* 2005, at 78.; Kheirabadi *et al.*, *J. Trauma* 59:25-35 (2005).

**[0013]** Other fibrinogen/thrombin-based dressings have also been proposed. For example, U.S. Patent No. 4,683,142 discloses a resorptive sheet material for closing and healing wounds which consists of a glycoprotein matrix, such as collagen, containing coagulation proteins, such as

fibrinogen and thrombin. U.S. Patent No. 5,702,715 discloses a reinforced biological sealant composed of separate layers of fibrinogen and thrombin, at least one of which also contains a reinforcement filler such as PEG, PVP, BSA, mannitol, FICOLL, dextran, myo-inositol or sodium chlorate. U.S. Patent No. 6,056,970 discloses dressings composed of a bioabsorbable polymer, such as hyaluronic acid or carboxymethylcellulose, and a haemostatic composition composed of powdered thrombin and/or powdered fibrinogen. U.S. Patent No. 7,189,410 discloses a bandage composed of a backing material having thereon: (i) particles of fibrinogen; (ii) particles of thrombin; and (iii) calcium chloride. U.S. Patent Application Publication No. US 2006/0155234 A1 discloses a dressing composed of a backing material and a plurality of fibrinogen layers which have discrete areas of thrombin between them. To date, none of these dressings have been approved for use or are available commercially.

[0014] Accordingly, there remains a need in the art for a solid dressing that can be used to treat wounded tissue, particularly wounded tissue resulting from traumatic injury in the field.

## SUMMARY OF THE INVENTION

[0015] It is therefore an object of the present invention to provide a solid dressing that can treat wounded mammalian tissue, particularly wounded tissue resulting from a traumatic injury. It is further an object of the present invention to provide a method of treating wounded mammalian tissue, particularly human tissue. Other objects, features and advantages of the present invention will be set forth in the detailed description of preferred embodiments that follows, and will in part be apparent from that description and/or may be learned by practice of the present invention. These objects and advantages will be realized and attained by the compositions and methods described in this specification and particularly pointed out in the claims that follow.

[0016] In accordance with these and other objects, a first embodiment of the present invention is directed to a solid dressing for treating wounded tissue in a mammal comprising at least one haemostatic layer consisting essentially of a fibrinogen component and thrombin, wherein the thrombin is present in an amount between about 0.250 Units/mg of fibrinogen component and 0.062 Units/mg of fibrinogen component.

[0017] Another embodiment is directed to a method of treating wounded tissue using a solid dressing comprising at least one haemostatic layer consisting essentially of a fibrinogen component and thrombin, wherein the thrombin is present in an amount between about 0.250 Units/mg of fibrinogen component and 0.062 Units/mg of fibrinogen component.

[0018] Other embodiments are directed to similar solid dressings wherein the amount of thrombin is between 0.125 Units/mg of fibrinogen component and 0.080 Units/mg of fibrinogen component., and the use of the same for treating wounded tissue.

[0019] It is to be understood that the foregoing general description and the following detailed description of preferred embodiments are exemplary and explanatory only and are intended to provide further explanation, but not limitation, of the invention as claimed herein.

## BRIEF DESCRIPTION OF DRAWINGS

[0020] **Figure 1A** and **Figure 1B** are graphs depicting the results of the EVPA and Adherence Assays.

[0021] **Figure 2** is a diagram of the set-up for the EVPA Assay.

**DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

[0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications mentioned herein are incorporated by reference.

[0023] As used herein, use of a singular article such as “a,” “an,” and “the” is not intended to excluded pluralities of the article’s object unless the context clearly and unambiguously dictates otherwise.

[0024] “Patient” as used herein refers to human or animal individuals in need of medical care and/or treatment.

[0025] “Wound” as used herein refers to any damage to any tissue of a patient which results in the loss of blood from the circulatory system and/or any other fluid from the patient’s body. The tissue may be an internal tissue, such as an organ or blood vessel, or an external tissue, such as the skin. The loss of blood may be internal, such as from a ruptured organ, or external, such as from a laceration. A wound may be in a soft tissue, such as an organ, or in hard tissue, such as bone. The damage may have been caused by any agent or source, including traumatic injury, infection or surgical intervention.

[0026] “Resorbable material” as used herein refers to a material that is broken down spontaneously in and/or by the mammalian body into components which are consumed or eliminated in such a manner as not to interfere significantly with wound healing and/or tissue regeneration, and without causing any significant metabolic disturbance.

[0027] “Stability” as used herein refers to the retention of those characteristics of a material that determine activity and/or function.

[0028] “Suitable” as used herein is intended to mean that a material does not adversely affect the stability of the dressings or any component thereof.

[0029] “Binding agent” as used herein refers to a compound or mixture of compounds that improves the adherence and/or cohesion of the components of the haemostatic layer(s) of the dressings.

[0030] “Solubilizing agent” as used herein refers to a compound or mixture of compounds that improves the dissolution of a protein or proteins in aqueous solvent.

[0031] “Filler” as used herein refers to a compound or mixture of compounds that provide bulk and/or porosity to the haemostatic layer(s) of a dressing.

[0032] “Release agent” as used herein refers to a compound or mixture of compounds that facilitates removal of a dressing from a manufacturing mold.

[0033] “Foaming agent” as used herein refers to a compound or mixture of compounds that produces gas when hydrated under suitable conditions.

[0034] “Solid” as used herein is intended to mean that the dressing will not substantially change in shape or form when placed on a rigid surface, wound-facing side down, and then left to stand at room temperature for 24 hours.

[0035] A first preferred embodiment of the present invention is directed to a solid dressing for treating wounded tissue in a patient which comprises a haemostatic layer consisting essentially of a fibrinogen component and thrombin, wherein the thrombin is present in an amount between 0.250 Units/mg of fibrinogen component and 0.062 Units/mg of fibrinogen component.

**[0036]** As used herein, “consisting essentially of” is intended to mean that the fibrinogen component and the thrombin are the only necessary and essential ingredients of the haemostatic layer(s) of the solid dressing when it is used as intended to treat wounded tissue. Accordingly, the haemostatic layer may contain other ingredients in addition to the fibrinogen component and the thrombin as desired for a particular application, but these other ingredients are not required for the solid dressing to function as intended under normal conditions, *i.e.* these other ingredients are not necessary for the fibrinogen component and thrombin to react and form enough fibrin to reduce the flow of blood and/or fluid from normal wounded tissue when that dressing is applied to that tissue under the intended conditions of use. If, however, the conditions of use in a particular situation are not normal, for example the patient is a hemophiliac suffering from Factor XIII deficiency, then the appropriate additional components, such as Factor XIII/XIIIa or some other transaminase, may be added to the haemostatic layer(s) without deviating from the spirit of the present invention. Similarly, the solid dressing of the present invention may contain one or more of these haemostatic layers as well as one or more other layers, such as one or more support layers (*e.g.* a backing material or an internal support material) and release layers.

**[0037]** Other preferred embodiments are directed to similar solid dressings wherein the amount of thrombin is between 0.125 Units/mg of fibrinogen component and 0.080 Units/mg of fibrinogen component. Still other preferred embodiments of the present invention are directed to similar solid dressings wherein the amount of thrombin is (all values being  $\pm 0.0009$ ): 0.250 Units/mg of fibrinogen component; 0.125 Units/mg of fibrinogen component; 0.100 Units/mg of fibrinogen component; 0.080 Units/mg of fibrinogen component; 0.062 Units/mg of fibrinogen component; 0.050 Units/mg of fibrinogen component; and 0.025 Units/mg of fibrinogen component.

**[0038]** Another preferred embodiment of the present invention is directed to a method for treating wounded tissue in a mammal, comprising placing a solid dressing of the present invention to wounded tissue and applying sufficient pressure to the dressing for a sufficient time for enough fibrin to form to reduce the loss of blood and/or other fluid from the wound.

**[0039]** According to certain embodiments of the present invention, the haemostatic layer(s) of the solid dressing is formed or cast as a single piece. According to certain other embodiments of the present invention, the haemostatic layer is made or formed into or from a single source, *e.g.* an aqueous solution containing a mixture of the fibrinogen component and the thrombin. With each of these embodiments of the present invention, the haemostatic layer(s) is preferably substantially homogeneous throughout.

**[0040]** According to other preferred embodiments, the haemostatic layer(s) of the solid dressing are composed of a plurality of particles, each of which consists essentially of a fibrinogen component and thrombin. According to such embodiments, the haemostatic layer may also contain a binding agent to facilitate or improve the adherence of the particles to one another and/or to any support layer(s). Illustrative examples of suitable binding agents include, but are not limited to, sucrose, mannitol, sorbitol, gelatin, maltose, hyaluron and its derivatives, such as hyaluronic acid, povidone, starch, chitosan and its derivatives (*e.g.* NOCC-chitosan), and cellulose derivatives, such as carboxymethylcellulose, as well as mixtures of two or more thereof.

**[0041]** The haemostatic layer(s) of the solid dressing may also optionally contain one or more suitable fillers, such as sucrose, lactose, maltose, silk, fibrin, collagen, albumin, polysorbate (Tween™), chitin, chitosan and its derivatives, such as NOCC-chitosan, alginic acid and salts thereof, hyaluron and its derivatives, such as hyaluronic acid, cellulose and derivatives thereof,

proteoglycans, glycolic acid polymers, lactic acid polymers, glycolic acid/lactic acid co-polymers, and mixtures of two or more thereof.

[0042] The haemostatic layer of the solid dressing may also optionally contain one or more suitable solubilizing agents, such as sucrose, dextrose, mannose, trehalose, mannitol, sorbitol, hyaluron and its derivatives, such as hyaluronic acid, albumin, sorbate, polysorbate (Tween™), sorbitan (SPAN™) and mixtures of two or more thereof.

[0043] The haemostatic layer of the solid dressing may also optionally contain one or more suitable foaming agents, such as a mixture of a physiologically acceptable acid (*e.g.* citric acid or acetic acid) and a physiologically suitable base (*e.g.* sodium bicarbonate or calcium carbonate). Other suitable foaming agents include, but are not limited to, dry particles containing pressurized gas, such as sugar particles containing carbon dioxide (*see, e.g.*, U.S. Patent No. 3,012,893) or other physiologically acceptable gases (*e.g.* Nitrogen or Argon), and pharmacologically acceptable peroxides.

[0044] The haemostatic layer(s) of the solid dressing may also optionally contain a suitable source of calcium ions, such as calcium chloride, and/or a fibrin cross-linker, such as a transaminase (*e.g.* Factor XIII/XIIIa) or glutaraldehyde.

[0045] The haemostatic layer of the solid dressing is preferably prepared by mixing aqueous solutions of the fibrinogen component and the thrombin under conditions which minimize the activation of the fibrinogen component by the thrombin. The mixture of aqueous solutions is then subjected to a process such as lyophilization or freeze-drying to reduce the moisture content to the desired level, *i.e.* to a level where the dressing is solid and therefore will not substantially change in shape or form upon standing, wound-facing surface down, at room temperature for 24 hours.

Similar processes that achieve the same result, such as drying, spray-drying, vacuum drying and vitrification, may also be employed.

[0046] As used herein, "moisture content" refers to the amount freely-available water in the dressing. "Freely-available" means the water is not bound to or complexed with one or more of the non-liquid components of the dressing. The moisture content referenced herein refers to levels determined by procedures substantially similar to the FDA-approved, modified Karl Fischer method (Meyer and Boyd, *Analytical Chem.*, 31:215-219, 1959; May *et al.*, *J. Biol. Standardization*, 10:249-259, 1982; Centers for Biologics Evaluation and Research, FDA, Docket No. 89D-0140, 83-93; 1990) or by near infrared spectroscopy. Suitable moisture content(s) for a particular solid dressing may be determined empirically by one skilled in the art depending upon the intended application(s) thereof.

[0047] For example, in certain embodiments of the present invention, higher moisture contents are associated with more flexible solid dressings. Thus, in solid dressings intended for extremity wounds, it may be preferred to have a moisture content of at least 6% and even more preferably in the range of 6% to 44%.

[0048] Similarly, in other embodiments of the present invention, lower moisture contents are associated with more rigid solid dressings. Thus, in solid dressings intended for flat wounds, such as wounds to the abdomen or chest, it may be preferred to have a moisture content of less than 6% and even more preferably in the range of 1% to 6%.

[0049] Accordingly, illustrative examples of suitable moisture contents for solid dressings include, but are not limited to, the following (each value being  $\pm 0.9\%$ ): less than 53%; less than 44%; less than 28%; less than 24%; less than 16%; less than 12%; less than 6%; less than 5%; less

than 4%; less than 3%; less than 2.5%; less than 2%; less than 1.4%; between 0 and 12%, non-inclusive; between 0 and 6%; between 0 and 4%; between 0 and 3%; between 0 and 2%; between 0 and 1%; between 1 and 16%; between 1 and 11%; between 1 and 8%; between 1 and 6%; between 1 and 4%; between 1 and 3%; between 1 and 2%; and between 2 and 4%.

[0050] The fibrinogen component in the haemostatic layer(s) of the solid dressings may be any suitable fibrinogen known and available to those skilled in the art. The fibrinogen component may also be a functional derivative or metabolite of a fibrinogen, such the fibrinogen  $\alpha$ ,  $\beta$  and/or  $\gamma$  chains, soluble fibrin I or fibrin II, or a mixture of two or more thereof. A specific fibrinogen (or functional derivative or metabolite) for a particular application may be selected empirically by one skilled in the art. As used herein, the term "fibrinogen" is intended to include mixtures of fibrinogen and small mounts of Factor XIII/Factor XIIIa, or some other such transaminase. Such small amounts are generally recognized by those skilled in the art as usually being found in mammalian fibrinogen after it has been purified according to the methods and techniques presently known and available in the art and typically range from 0.1 to 20 Units/mL.

[0051] Preferably, the fibrinogen employed as the fibrinogen component of the solid dressing is a purified fibrinogen suitable for introduction into a mammal. Typically, such fibrinogen is a part of a mixture of human plasma proteins which include Factor XIII/XIIIa and have been purified to an appropriate level and virally inactivated. A preferred aqueous solution of fibrinogen for preparation of a solid dressing contains around 37.5 mg/mL fibrinogen at a pH of around  $7.4 \pm 0.1$ , although a pH in the range of 5.5-8.5 may be acceptable. Suitable fibrinogen for use as the fibrinogen component has been described in the art, *e.g.* U.S. Patent No. 5,716,645, and similar materials are commercially available, *e.g.* from sources such as Sigma-Aldrich, Enzyme Research Laboratories, Haematologic Technologies and Anlara.

[0052] Preferably, the fibrinogen component is present in an amount of from about 1.5 to about 13.0 mg ( $\pm 0.9$  mg) of fibrinogen per square centimeter of solid dressing, and more preferably from about 3.0 to about 13.0 mg/cm<sup>2</sup>. Greater or lesser amounts, however, may be employed depending upon the particular application intended for the solid dressing. For example, according to certain embodiments where increased adherence is desired, the fibrinogen component is present in an amount of from about 11.0 to about 13.0 mg ( $\pm 0.9$  mg) of fibrinogen per square centimeter of solid dressing. Likewise, according to certain embodiments which are intended for treating low pressure-containing vessels, lower levels of the fibrinogen component may be employed.

[0053] While any suitable mammalian thrombin may be used in a solid dressing, the thrombin employed in the haemostatic layer is preferably a lyophilized mixture of human plasma proteins which has been sufficiently purified and virally inactivated for the intended use of the solid dressing. Suitable thrombin is available commercially from sources such as Sigma-Aldrich, Enzyme Research Laboratories, Haematologic Technologies and Biomol International. A particularly preferred aqueous solution of thrombin for preparing a solid dressing contains thrombin at a potency of between 10 and 2000  $\pm 50$  International Units/mL, and more preferred at a potency of 25  $\pm 2.5$  International Units/mL. Other constituents may include albumin (generally about 0.1 mg/mL) and glycine (generally about 100 mM  $\pm 0.1$  mM). The pH of this particularly preferred aqueous solution of thrombin is generally in the range of 6.5-7.8, and preferably 7.4  $\pm 0.1$ , although a pH in the range of 5.5-8.5 may be acceptable.

[0054] In addition to the haemostatic layer(s), the solid dressing may optionally further comprise one or more support layers. As used herein, a "support layer" refers to a material that sustains or improves the structural integrity of the solid dressing and/or the fibrin clot formed when such a dressing is applied to wounded tissue.

[0055] According to certain preferred embodiments of the present invention the support layer comprises a backing material on the side of the dressing opposite the side to be applied to wounded tissue. Such a backing material may be affixed with a physiologically-acceptable adhesive or may be self-adhering (*e.g.* by having a sufficient surface static charge). The backing material may comprise one or more resorbable materials or one or more non-resorbable materials or mixtures thereof. Preferably, the backing material is a single resorbable material.

[0056] Any suitable resorbable material known and available to those skilled in the art may be employed in the present invention. For example, the resorbable material may be a proteinaceous substance, such as silk, fibrin, keratin, collagen and/or gelatin. Alternatively, the resorbable material may be a carbohydrate substance, such as alginates, chitin, cellulose, proteoglycans (*e.g.* poly-N-acetyl glucosamine), hyaluron and its derivatives, such as hyaluronic acid, glycolic acid polymers, lactic acid polymers, or glycolic acid/lactic acid co-polymers. The resorbable material may also comprise a mixture of proteinaceous substances or a mixture of carbohydrate substances or a mixture of both proteinaceous substances and carbohydrate substances. Specific resorbable material(s) may be selected empirically by those skilled in the art depending upon the intended use of the solid dressing.

[0057] According to certain preferred embodiments of the present invention, the resorbable material is a carbohydrate substance. Illustrative examples of particularly preferred resorbable materials include, but are not limited to, the materials sold under the trade names VICRYL™ (a glycolic acid/lactic acid copolymer) and DEXON™ (a glycolic acid polymer).

[0058] Any suitable non-resorbable material known and available to those skilled in the art may be employed as the backing material. Illustrative examples of suitable non-resorbable materials

include, but are not limited to, plastics, silicone polymers, gauze, latexes, paper and paper products, and the like.

**[0059]** According to other preferred embodiments, the support layer comprises an internal support material. Such an internal support material is preferably fully embedded or contained within a haemostatic layer of the solid dressing, although it may be placed between two adjacent haemostatic layers in certain embodiments. As with the backing material, the internal support material may be a resorbable material or a non-resorbable material, or a mixture thereof, such as a mixture of two or more resorbable materials or a mixture of two or more non-resorbable materials or a mixture of resorbable material(s) and non-resorbable material(s).

**[0060]** According to still other preferred embodiments, the support layer may comprise a front support material on the wound-facing side of the dressing, *i.e.* the side to be applied to wounded tissue. As with the backing material and the internal support material, the front support material may be a resorbable material or a non-resorbable material, or a mixture thereof, such as a mixture of two or more resorbable materials or a mixture of two or more non-resorbable materials or a mixture of resorbable material(s) and non-resorbable material(s).

**[0061]** According to still other preferred embodiments, the solid dressing comprises both a backing material and an internal support material in addition to the haemostatic layer(s), *i.e.* the solid dressing comprises two support layers in addition to the haemostatic layer(s). According to still other preferred embodiments, the solid dressing comprises both a front support material and an internal support material in addition to the haemostatic layer(s). According to still other preferred embodiments, the solid dressing comprises a backing material, a front support material and an internal support material in addition to the haemostatic layer(s).

[0062] According to certain embodiments of the present invention, particularly where the dry dressing is manufactured using a mold, the dry dressings may also optionally further comprise a release layer in addition to the hemostatic layer(s) and support layer(s). As used herein, a “release layer” refers to a layer containing one or more agents (“release agents”) which promote or facilitate removal of the dry dressing from a mold in which it has been manufactured. A preferred such agent is sucrose, but other suitable release agents include gelatin, mannitol, sorbitol, polysorbate (Tween™), sorbitan (SPAN™), lactose, maltose, trehalose, hyaluron and its derivatives, such as hyaluronic acid, sorbate, glucose and mixtures of two or more thereof. Alternatively, such one or more release agents may be contained in the hemostatic layer.

[0063] The various layers of the inventive dressings may be affixed to one another by any suitable means known and available to those skilled in the art. For example, a physiologically-acceptable adhesive may be applied to a backing material (when present), and the haemostatic layer(s) subsequently affixed thereto.

[0064] In certain embodiments of the present invention, the physiologically-acceptable adhesive has a shear strength and/or structure such that the backing material can be separated from the fibrin clot formed by the haemostatic layer after application of the dressing to wounded tissue. In other embodiments, the physiologically-acceptable adhesive has a shear strength and/or structure such that the backing material cannot be separated from the fibrin clot after application of the bandage to wounded tissue.

[0065] Suitable fibrinogen components and suitable thrombin for the haemostatic layer(s) of the solid dressing may be obtained from any appropriate source known and available to those skilled in the art, including, but not limited to, the following: from commercial vendors, such as Sigma-Aldrich and Enzyme Research Laboratories; by extraction and purification from human or

mammalian plasma by any of the methods known and available to those skilled in the art; from supernatants or pastes derived from plasma or recombinant tissue culture, viruses, yeast, bacteria, or the like that contain a gene that expresses a human or mammalian plasma protein which has been introduced according to standard recombinant DNA techniques; and/or from the fluids (*e.g.* blood, milk, lymph, urine or the like) of transgenic mammals (*e.g.* goats, sheep, cows) that contain a gene which has been introduced according to standard transgenic techniques and that expresses the desired fibrinogen component and/or desired thrombin.

**[0066]** According to certain preferred embodiments of the present invention, the fibrinogen component is a mammalian fibrinogen such as bovine fibrinogen, porcine fibrinogen, ovine fibrinogen, equine fibrinogen, caprine fibrinogen, feline fibrinogen, canine fibrinogen, murine fibrinogen or human fibrinogen. According to other embodiments, the fibrinogen component is bird fibrinogen or fish fibrinogen. According to still other embodiments, the fibrinogen component is human fibrinogen, human fibrinogen  $\alpha$  chain, human fibrinogen  $\beta$  chain, human fibrinogen  $\gamma$  chain, human fibrin I, human fibrin II, or a mixture of two or more thereof. According to any of these embodiments, the fibrinogen may be recombinantly produced fibrinogen or transgenic fibrinogen. As noted above, the fibrinogen may also contain small amounts (*e.g.* \_\_\_ % of total protein) of a transaminase, such as Factor XIII/XIIIa.

**[0067]** According to certain preferred embodiments of the present invention, the thrombin is a mammalian thrombin, such as bovine thrombin, porcine thrombin, ovine thrombin, equine thrombin, caprine thrombin, feline thrombin, canine thrombin, murine thrombin and human thrombin. According to other embodiments, the thrombin is bird thrombin or fish thrombin. According to any of these embodiments, the thrombin may be recombinantly produced thrombin or transgenic thrombin.

[0068] As a general proposition, the purity of the fibrinogen component and/or the thrombin for use in the solid dressing will be a purity known to one of ordinary skill in the relevant art to lead to the optimal efficacy and stability of the protein(s). Preferably, the fibrinogen component and/or the thrombin has been subjected to multiple purification steps, such as precipitation, concentration, diafiltration and affinity chromatography (preferably immunoaffinity chromatography), to remove substances which cause fragmentation, activation and/or degradation of the fibrinogen component and/or the thrombin during manufacture, storage and/or use of the solid dressing. Illustrative examples of such substances that are preferably removed by purification include: protein contaminants, such as inter-alpha trypsin inhibitor and pre-alpha trypsin inhibitor; non-protein contaminants, such as lipids; and mixtures of protein and non-protein contaminants, such as lipoproteins.

[0069] The amount of the fibrinogen component employed in the solid dressing is preferably selected to optimize both the efficacy and stability thereof. As such, a suitable concentration for a particular application of the solid dressing may be determined empirically by one skilled in the relevant art. According to certain preferred embodiments of the present invention, when the fibrinogen component is human fibrinogen, the amount of fibrinogen employed is between 1.5 mg and 13.0 mg (each  $\pm$  0.9 mg) per square centimeter of solid dressing, more preferably between 3.0 mg and 13.0 mg per square centimeter and most preferably between 11.0 mg and 13.0 mg per square centimeter.

[0070] During use of the solid dressing, the fibrinogen component and the thrombin are preferably activated at the time the dressing is applied to the wounded tissue by the endogenous fluids of the patient escaping from the hemorrhaging wound. Alternatively, in situations where fluid loss from the wounded tissue is insufficient to provide adequate hydration of the protein layers, the

fibrinogen component and/or the thrombin may be activated by a suitable, physiologically-acceptable liquid, optionally containing any necessary co-factors and/or enzymes, prior to or during application of the dressing to the wounded tissue.

**[0071]** In some embodiments of the present invention, the haemostatic layer(s) may also contain one or more supplements, such as growth factors, drugs, polyclonal and monoclonal antibodies and other compounds. Illustrative examples of such supplements include, but are not limited to, the following: fibrinolysis inhibitors, such as aprotonin, tranexamic acid and epsilon-amino-caproic acid; antibiotics, such as tetracycline and ciprofloxacin, amoxicillin, and metronidazole; anticoagulants, such as activated protein C, heparin, prostacyclins, prostaglandins (particularly (PGI<sub>2</sub>), leukotrienes, antithrombin III, ADPase, and plasminogen activator; steroids, such as dexamethasone, inhibitors of prostacyclin, prostaglandins, leukotrienes and/or kinins to inhibit inflammation; cardiovascular drugs, such as calcium channel blockers, vasodilators and vasoconstrictors; chemoattractants; local anesthetics such as bupivacaine; and antiproliferative/antitumor drugs such as 5-fluorouracil (5-FU), taxol and/or taxotere; antivirals, such as gangcyclovir, zidovudine, amantidine, vidarabine, ribaravin, trifluridine, acyclovir, dideoxyuridine and antibodies to viral components or gene products; cytokines, such as alpha- or beta- or gamma-Interferon, alpha- or beta-tumor necrosis factor, and interleukins; colony stimulating factors; erythropoietin; antifungals, such as diflucan, ketaconizole and nystatin; antiparasitic gents, such as pentamidine; anti-inflammatory agents, such as alpha-1-anti-trypsin and alpha-1-antichymotrypsin; anesthetics, such as bupivacaine; analgesics; antiseptics; hormones; vitamins and other nutritional supplements; glycoproteins; fibronectin; peptides and proteins; carbohydrates (both simple and/or complex); proteoglycans; antiangiogenins; antigens; lipids or liposomes; oligonucleotides (sense and/or antisense DNA and/or RNA); and gene therapy reagents. In other embodiments of the present invention, the backing layer and/or the internal support layer,

if present, may contain one or more supplements. According to certain preferred embodiments of the present invention, the therapeutic supplement is present in an amount greater than its solubility limit in fibrin.

[0072] The following examples are illustrative only and are not intended to limit the scope of the invention as defined by the appended claims. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods of the present invention without departing from the spirit and scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

## EXAMPLES

[0073] The ability of the dressings to seal an injured blood vessel was determined by an *ex vivo* porcine arteriotomy (EVPA) performance test, which was first described in U.S. Patent No. 6,762,336. The EVPA performance test evaluates the ability of a dressing to stop fluid flow through a hole in a porcine artery. While the procedure described in U.S. Patent No. 6,762,336 has been shown to be useful for evaluating haemostatic dressings, it failed to replicate faithfully the requirements for success *in vivo*. More specifically, the procedure disclosed in U.S. Patent No. 6,762,336 required testing at 37°C, whereas, in the real world, wounds are typically cooler than that. This decreased temperature can significantly reduce the rate of fibrin formation and its haemostatic efficacy in trauma victims. *See, e.g., Acheson et al., J. Trauma 59:865-874 (2005)*. The test in U.S. Patent No. 6,762,336 also failed to require a high degree of adherence of the dressing to the injured tissue. A failure mode in which fibrin forms but the dressing fails to attach tightly to the tissue would, therefore, not be detected by this test. Additionally, the pressure utilized in the procedure

(200mmHg) may be exceeded during therapy for some trauma patients. The overall result of this is that numerous animal tests, typically involving small animals (such as rats and rabbits), must be conducted to accurately predict dressing performance in large animal, realistic trauma studies and in the clinical environment.

[0074] In order to minimize the amount of time and the number of animal studies required to develop the present invention, an improved *ex vivo* testing procedure was developed. To accomplish this, the basic conditions under which the dressing test was conducted were changed, and the severity of the test parameters was increased to include testing at lower temperatures (*i.e.* 29-33°C vs. 37 °C, representing the real physiologic challenge at realistic wound temperatures (Acheson *et al.*, J. Trauma 59:865-874 (2005)), higher pressures (*i.e.* 250 mmHg vs. 200 mmHg), a longer test period (3 minutes vs. 2 minutes) and larger sized arterial injuries (U.S. Patent No. 6,762,336 used an 18 gauge needle puncture, whereas the revised procedure used puncture holes ranging from 2.8mm to 4mm x 6mm).

[0075] In addition, a new test was derived to directly measure adherence of the dressing to the injured tissue. Both these tests showed greatly improved stringency and are thus capable of surpassing the previous *ex vivo* test and replacing many *in vivo* tests for efficacy.

[0076] The following is a list of acronyms used in the Examples below:

- CFB: Complete Fibrinogen Buffer (100mM Sodium Chloride, 1.1mM Calcium Chloride, 10mM Tris, 10mM Sodium Citrate, 1.5% Sucrose, Human Serum Albumin (80mg/g of total protein) and Tween™ 80 (animal source) 15mg/g total protein)
- CTB: Complete Thrombin Buffer (150mM Sodium Chloride, 40mM Calcium Chloride, 10mM Tris and 100mM L-Lysine with the addition of HSA at 100ug/ml)

- ERL: Enzyme Research Laboratories
- EVPA: *Ex Vivo* Porcine Arteriotomy
- FD: Inventive haemostatic dressing
- HSA: Human Serum Albumin
- HD: A "sandwich" fibrin sealant haemostatic dressing as disclosed in U.S. Patent No. 6,762,336
- IFB: Incomplete Fibrinogen Buffer.; CFB without HSA and Tween
- PETG: Glycol-modified Polyethylenetetrathalate
- PPG: Polypropylene
- PVC: Poly vinyl chloride
- TRIS: trishydroxymethylaminomethane (2-amino-2-hydroxymethyl-1,3-propanediol)

### Example 1

[0077] Backing material (DEXON™) was placed into 2.4 X 2.4cm PETG molds. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. Once completed the molds were placed in a -80°C freezer for at least 60 minutes.

[0078] Fibrinogen (Enzyme Research Laboratories™ (ERL) lot 3114) was formulated in 100mM Sodium Chloride, 1.1mM Calcium Chloride, 10mM Tris, 10mM Sodium Citrate, 1.5% Sucrose, Human Serum Albumin (HSA) at a concentration of 80mg/g of total protein and 15mg/g total protein of Tween™ 80 (animal source) (Complete Fibrinogen buffer (CFB)). The fibrinogen concentration was adjusted to 37.5mg/ml using CFB. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . Once prepared the fibrinogen was placed on ice until use.

[0079] Thrombin was formulated in 150mM Sodium Chloride, 40mM Calcium Chloride, 10mM Tris and 100mM L-Lysine with the addition of HSA at 100ug/ml (Complete Thrombin

Buffer (CTB)). The final pH of the thrombin was  $7.4 \pm 0.1$ . The thrombin concentrations were adjusted with CFB to produce 12.5 units/mg of Fibrinogen (upon mixing), which corresponded to 3120 Units/ml thrombin prior to mixing. Once prepared the thrombin was placed on ice until use.

[0080] The temperature of the fibrinogen and thrombin prior to dispensing was  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Molds were removed from the  $-80^{\circ}\text{C}$  freezer and placed on a copper plate that was precooled on top of dry ice. A repeat pipettor was filled with fibrinogen and second repeat pipettor was filled with thrombin. Two ml of fibrinogen and 300 micro liters of thrombin were dispensed simultaneously into each mold. Once the molds were filled they were returned to the  $-80^{\circ}\text{C}$  freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

## Example 2

[0081] Backing material was placed into each 1.5 X 1.5cm PVC molds. Fifteen microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. A second piece of PETG plastic was fitted on top of the 1.5 X 1.5 molds and held in place. This formed a closed mold. The molds were then placed in a  $-80^{\circ}\text{C}$  freezer for at least 60 minutes. Fibrinogen (ERL lot 3100) was formulated in CFB. The fibrinogen concentration was adjusted to 37.5mg/ml using CFB. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . Once prepared the fibrinogen was placed on ice until use. Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . The thrombin concentrations were adjusted using CTB to deliver the following amounts 2.5, 0.25, 0.1, 0.05, 0.025, 0.016, 0.0125 and 0.01 units/mg of Fibrinogen (upon mixing), which corresponded to 624, 62.4, 25, 12.5, 6.24, 3.99, 3.12, and 2.5 Units/ml thrombin prior to mixing. Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Molds were then removed from the  $-80^{\circ}\text{C}$  freezer and placed on an

aluminum plate that was pre-cooled on top of dry ice. Three holes were punched at the top of the mold using an 18 gauge needle. One hole was used for injecting fibrinogen, the second for injecting thrombin, and the third hole served as a vent to release air that was displaced from inside the mold. A pipette was then filled with fibrinogen and a second pipette with thrombin. Simultaneously 0.78ml of fibrinogen and 0.17ml of thrombin were injected via these pipettes into each mold. Once filled the molds were placed on top of a pool of liquid nitrogen for thirty seconds and then returned to the -80°C freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

### Example 3

[0082] Backing material was placed into 2.4 X 2.4cm PVC molds. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. Once completed the molds were placed in a -80°C freezer for at least 60 minutes. Fibrinogen (ERL lot 3100) was formulated in CFB. The fibrinogen concentration was adjusted to 37.5mg/ml using CFB. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . Once prepared the fibrinogen was placed on ice until use. Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . Using CTB, the thrombin concentrations were adjusted to deliver the following amounts 0.125, 0.025, 0.0125, 0.00625 and 0.0031 units/mg of Fibrinogen upon mixing, which corresponded to 31.2, 6.24, 3.12, 1.56 and 0.78 Units/ml thrombin prior to mixing. Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^\circ\text{C} \pm 2^\circ\text{C}$ . The molds were removed from the -80°C freezer and placed on an aluminum plate that that was precooled on top of dry ice. A 3ml syringe fitted with an 18 gauge needle was filled with 2ml of fibrinogen and a second, 1ml, syringe fitted with a 22 gauge needle was filled with 0.3ml of

thrombin. The contents of both syringes were dispensed simultaneously into each mold. Once filled the molds were placed on top of liquid nitrogen for thirty seconds and then returned to the -80°C freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

#### Example 4

[0083] Backing material was placed into PVC 2.4 X 2.4cm molds. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. Once completed the molds were placed in a -80°C freezer for at least 60 minutes. A vial containing 3 grams of Fibrinogen (Sigma™ Lot# F-3879) was removed from the -20°C freezer and placed at 4°C for 18 hours. The bottle was then removed from the freezer and allowed to come to room temperature for 60 minutes. To the bottle, 60ml of 37°C water was added and allowed to mix for 15 minutes at 37°C. Once in solution the fibrinogen was dialyzed against incomplete fibrinogen buffer (IFB, which was CFB without HSA and Tween™) for 4 hours at room temperature. At the end of the four hours HSA was added to a concentration of 80mg/g of total protein, and Tween™ 80 (animal source) was added to a concentration of 15mg/g total protein. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . The fibrinogen concentration was then adjusted to 37.5mg/ml with CFB. Once prepared the fibrinogen was placed on ice until use. Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . Using CTB, the thrombin concentrations were adjusted to deliver the following amounts 2.5, 0.25, 0.125, 0.083 and 0.0625 units/mg of Fibrinogen (upon mixing) which corresponded to 624, 62.4, 31.2, 20.8 and 15.6 Units/ml thrombin prior to mixing. Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^\circ\text{C} \pm 2^\circ\text{C}$ . Molds were removed from the -80°C freezer and placed on an aluminum

plate that was that was precooled on top of dry ice. A 3ml syringe fitted with an 18 gauge needle was filled with 2ml of fibrinogen and a second 1ml syringe fitted with a 22 gauge needle was filled with 0.3ml of thrombin. The contents of both syringes were dispensed simultaneously into each mold. Once filled the molds were placed on top of liquid nitrogen for thirty seconds and then returned to the -80°C freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

#### Example 5

[0084] Backing material was placed into 2.4 X 2.4cm PVC molds. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. A second piece of PETG plastic was cut to fit on top of the molds and held in place by clips located at each end of the mold, producing closed molds. Once completed the molds were placed in a -80°C freezer for at least 60 minutes. Fibrinogen (ERL lot 3060 was formulated in CFB. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . The fibrinogen concentration was adjusted to 37.5mg/ml using CFB. Once prepared the fibrinogen was placed on ice until use. Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . Using CTB, thrombin concentrations were adjusted to deliver the following amounts 2.5, 0.25, 0.125, 0.083 and 0.062 units/mg of Fibrinogen (after mixing), which corresponded to 624, 62.4, 31.2, 20.8, and 15.6 Units/ml thrombin (prior to mixing). Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Molds were removed from the -80°C freezer and placed on an aluminum plate that was that was precooled on top of dry ice. A 3ml syringe fitted with an 18 gauge needle was filled with 2ml of fibrinogen and a second, 1ml, syringe fitted with a 22 gauge needle was filled with 0.3ml of thrombin. The contents of both syringes were dispensed

simultaneously into each mold. Once filled the molds were placed on top of liquid nitrogen for thirty seconds and then returned to the -80°C freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

### Example 6

[0085] Backing material was placed into 2.4 X 2.4cm PVC molds. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. A second piece of PETG plastic was cut to fit on top of the 2.4 X 2.4 molds and held in place by the use of clips located at each end of the mold to create closed molds. The molds were then placed in a -80°C freezer for at least 60 minutes. A vial containing 3 grams of Fibrinogen (Sigma Lot# F-3879) was removed from the -20°C freezer and placed at 4°C for 18 hours. The bottle was then removed from the freezer and allowed to come to room temperature for 60 minutes. To the bottle, 60ml of 37°C water was added and allowed to mix for 15 minutes at 37°C. Once in solution the fibrinogen was dialyzed against IFB. At the end of the four hours HSA was added to a concentration of 80mg/g of total protein, and Tween™ 80 (animal source) was added to a concentration of 15mg/g total protein. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . The fibrinogen concentration was adjusted to 37.5mg/ml using CFB. Once prepared the fibrinogen was placed on ice until use. Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . Thrombin concentration was adjusted to deliver the following amounts 2.5, 0.25, 0.125, 0.1 and 0.083 units/mg of Fibrinogen (upon mixing), which corresponded to 624, 62.4, 31.2, 24.96 and 20.79 Units/ml thrombin (before mixing). Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^\circ\text{C} \pm 2^\circ\text{C}$ . Molds were removed from the -80°C freezer and placed on an aluminum plate that was that was precooled on top of dry ice. A 3ml

syringe fitted with an 18 gauge needle was filled with 2ml of fibrinogen and a second, 1ml, syringe fitted with a 22 gauge needle was filled with 0.3ml of thrombin. The contents of both syringes were dispensed simultaneously into each mold. Once filled the molds were placed on top of liquid nitrogen for thirty seconds and then returned to the -80°C freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

### Example 7

[0086] Backing material was placed into 2.4 x 2.4cm PVC molds. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. A second piece of PETG plastic was cut to fit on top of the molds and held in place by the use of clips located at each end of the mold to create closed molds. Once completed, the molds were placed in a -80°C freezer for at least 60 minutes.

[0087] A vial containing 3 grams of Fibrinogen (Sigma™ Lot# F-3879) was removed from the -20°C freezer and placed at 4°C for 18 hours. The bottle was then allowed to come to room temperature for 60 minutes. To the bottle, 60ml of 37°C water was added and allowed to mix for 20 minutes at 37°C. Once in solution, the fibrinogen was dialyzed against IFB. At the end of the four hours, human serum albumin (HSA) was added to a concentration of 80mg/g of total protein, and Tween™ 80 (animal source) was added to a concentration of 15mg/g total protein. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . The fibrinogen concentration was adjusted to 37.5mg/ml using CFB. Once prepared the fibrinogen was placed on ice until use.

[0088] Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . Thrombin was adjusted to deliver the following amounts 2.5, 0.25, 0.125, 0.08 and 0.06 units/mg of

Fibrinogen (after mixing), which corresponded to 624, 62.4, 31.2, 20.8 and 15.6 Units/ml thrombin (prior to mixing). Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Molds were removed from the  $-80^{\circ}\text{C}$  freezer and placed on an aluminum plate that was that was precooled on top of dry ice. A 3ml syringe fitted with an 18 gauge needle was filled with 2ml of fibrinogen and a second, 1ml, syringe fitted with a 22 gauge needle was filled with 0.3ml of thrombin. The contents of both syringes were dispensed simultaneously into each mold. Once filled the molds were placed on top of liquid nitrogen for thirty seconds and then returned to the  $-80^{\circ}\text{C}$  freezer for at least two hours before being placed into the freeze dryer. They were then lyophilized as described below, and performance tested using the EVPA and Adherence Assays as described below.

### **Trilayer (Sandwich) Dressings**

[0089] Trilayer dressings were produced using the process described in US Patent 6,762,336, using the same sources of fibrinogen and thrombin as utilized to produce the monolithic dressings above.

### **Results**

[0090] The results of the EVPA and Adherence Assays are shown in Figures 1A and 1B, respectively.

### **Conclusions:**

[0091] Dressings produced with between 2.5 to 0.025 Thrombin Units/mg of Fibrinogen were active in both assays, while those with greater or lesser ratios of thrombin to fibrinogen were

not. Significantly greater activity was seen over the range of 2.5 to 0.05 Thrombin Units/mg of Fibrinogen. Greatly improved performance was seen between the ranges of 0.25 to 0.062 Thrombin Units/mg of Fibrinogen, while optimum performance was seen between the ranges of 0.125 to 0.08 Thrombin Units/mg of Fibrinogen. This contrasted with the dressings produced using the process described in U.S. Patent No. 6,762,336 which reached full performance at 12.5 Thrombin Units/mg of Fibrinogen, with unacceptable performance occurring as the thrombin concentration was diminished below 12.5 Thrombin Units/mg of Fibrinogen, with essentially no activity remaining at 1.4 Thrombin Units/mg of Fibrinogen. This difference in both the limits of performance and the optimum levels is all the more profound given that the performance of the Trilayer dressings from U.S. Patent No. 6,762,336 was decreased by the use of decreasing amounts of thrombin, while the dressing described herein showed an increased activity over this range.

#### Example 8

[0092] Monolithic dressings were manufactured as follows: backing material was cut and placed into each PETG 2.4 X 2.4cm mold. Twenty-five microliters of 2% sucrose was pipetted on top of each of the four corners of the backing material. Once completed the molds were placed in a -80°C freezer for at least 60 minutes.

[0093] For all dressings, ERL fibrinogen lot 3114 was formulated in CFB. The final pH of the fibrinogen was  $7.4 \pm 0.1$ . The fibrinogen concentration was adjusted to 37.5mg/ml. Once prepared the fibrinogen was placed on ice until use. Thrombin was formulated in CTB. The final pH of the thrombin was  $7.4 \pm 0.1$ . The thrombin was adjusted to deliver 0.1 units/mg of Fibrinogen or 25 Units/ml thrombin. Once prepared the thrombin was placed on ice until use. The temperature of the fibrinogen and thrombin prior to dispensing was  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Molds were

removed from the -80°C freezer and placed on a copper plate that was placed on top of dry ice. A repeat pipettor was filled with fibrinogen and second repeat pipettor was filled with thrombin. Simultaneously 2ml of fibrinogen and 300 micro liters of thrombin were dispensed into each mold. Once the molds were filled they were returned to the -80°C freezer for at least two hours before being placed into the freeze dryer. Dressings were then lyophilized as described above. Once complete the dressings were stored in low moisture transmission foil bags containing 5 grams of desiccant.

[0094] Trilayer dressings were manufactured as described previously<sup>1</sup>, using the same materials as described above. Subsequently, the dressings were placed under conditions of 100% relative humidity at 37°C for various times in order to increase their relative moisture content to desired levels. The dressings were evaluated visually and for their handling and other physical characteristics. Following this evaluation, a sample of each of the dressings was tested to determine their moisture content. The remaining dressings were performance tested in the EVPA, Adherence and Weight Held assays.

## Results

[0095] The results of the assays are given in the Tables below:

**Table 1: Performance Data of Inventive Solid Dressings**

Exposure Time to 100% Humidity @ 37°C (minutes)	% Moisture	EVPA # Pass/Total	Peel Test Adherence (± Std. Dev.)	Weight Held (g) (mean ± Std. Dev.)
0	2.5	2/2	4.0 ± 0	148 ± 28.3
1	5.8	2/2	3.5 ± 0.71	123 ± 7.1
15	16	2/2	2.5 ± .71	108 ± 14.1
45	24	2/2	4.0 ± 0	168 ± 0
60	28	2/2	4.0 ± 0	273 ± 7.1
225	44	2/2	2 ± 0	58 ± 14.1
1200	52	ND	ND	ND

**Table 2: Performance Data for Tri-layer Dressings**

Exposure Time to 100% Humidity @ 37°C (minutes)	% Moisture	EVPA # Pass/Total	Peel Test Adherence	Weight Held (g) (mean)
0	3	1/1	2.0	78
15	22	1/1	2.0	78
60	33.7	0/1	0.5	28

**Table 3: Integrity and Handling Characteristics of Inventive Solid Dressings**

Exposure Time to 100% Humidity @ 37°C (minutes)	Prior To Hydration				During Hydration		After Hydration
	Surface Appearance	Curling	Integrity	Flexible	Speed of Hydration	Force Required for Hydration	Appearance
0	Normal (Smooth, No "skin")	No	Excellent (No cracks or flaking off)	No	Normal	No	Normal
1	"	"	"	Yes	"	"	"
15	"	"	"	"	"	"	"
45	"	"	"	"	"	"	"
60	"	Slight	"	"	"	"	"
225	"	Yes	"	"	"	"	"
1200	"	Curling Up on Itself	"	"	n/d	n/d	Mottled & lumpy

**Table 4: Integrity and Handling Characteristics of Trilayer Dressings**

Exposure Time to 100% Humidity @ 37°C (minutes)	Prior To Hydration				During Hydration		After Hydration
	Surface Appearance	Curling	Integrity	Flexibility	Speed of Hydration	Force Required for Hydration	Appearance
0	Normal	No	Good. Some delamination	No	Normal	No	Normal
15	Irregular	No	"	Yes	Slow	No	Mottled
60	Skinned	Yes	"	Yes	Very Slow	Yes	Very Mottled and lumpy

**Conclusions:**

[0096] The monolithic dressings were fully functional at very high levels of moisture. As much as 28% moisture was found to retain complete functionality. When the moisture levels rose to 44%, the dressings were still functional, however some of their activity was reduced. Higher levels of moisture may also retain some function. The original dressings, at 2.5% moisture content, were not flexible, but had all the other desired properties including appearance, a flat surface, integrity, rapid and uncomplicated hydration and a smooth appearance post hydration. Once the moisture content was increased to 5.8%, the monolithic dressings became flexible, while retaining their functionality and desirable characteristics. They retained their flexibility, without curling or losing their integrity or appearing to form excessive amounts of fibrin prior to hydration.

[0097] This contrasted with the tri-layer dressings, which began to lose their desirable characteristics upon the addition of moisture, and lost them entirely by the time moisture had increased to 33%.

**Lyophilization Procedure**

[0098] Frozen dressings were placed into a pre-cooled Genesis™ lyophilizer (Virtis, Gardiner, NY). The chamber was sealed and the temperature equilibrated. The chamber was then evacuated and the dressings lyophilized via a primary and secondary drying cycle.

**EVPA Performance Testing****[0099] *Equipment and Supplies:***

- In-line high pressure transducer(Ashcroft Duralife™ or equivalent)

- Peristaltic pump (Pharmacia Biotech™, Model P-1 or equivalent)
- Voltmeter (Craftsman™ Professional Model 82324 or equivalent)
- Computer equipped with software for recording pressure or voltage information
- Tygon™ tubing (assorted sizes) with attachments
- Water bath (Baxter Durabath™ or equivalent), preset to 37°C
- Incubation chamber (VWR™, Model 1400G or equivalent), preset to 37°C
- Thermometer to monitor temperatures of both water bath and oven
- Assorted forceps, hemostats, and scissors
- 10cc. and 20cc. syringes with an approximately 0.6 cm hole drilled in center and smaller hole drilled through both syringe and plunger. This hole, drilled into the end of the syringe, will be used to keep the plunger drawn back and stationary.
- O-rings (size 10 and 13)
- Plastic Shields to fit the 10cc and 20cc syringes (approximately 3.5 cm in length)
- P-1000 Pipetman™ with tips
- Sphygmomanometer with neonatal size cuff and bladder
- Programmable Logic Controller (PLC) to control the pumps to maintain the desired pressure profile (Optional. Manual control may be used if desired.)

## **1. Materials and Chemicals**

- Porcine descending aortas (Pel-Freez Biologicals™, Catalog # 59402-2 or equivalent)
- Cyanoacrylate glue (Vetbond™, 3M or equivalent)
- 18-gauge needle(s)
- 0.9% Saline, maintained at 37°C

- Red food coloring
- Vascular Punch(es), 2.8mm or other
- Plastic Wrap

## **2. Artery Cleaning and Storage**

1. Store arteries at -20°C until used.
2. Thaw arteries at 37°C in H<sub>2</sub>O bath.
3. Clean fat and connective tissue from exterior surface of artery.
4. Cut the arteries into ~5cm segments.
5. The arteries may be refrozen to -20°C and stored until use.

## **3. Artery Preparation for Assay**

1. Turn the artery inside-out so that the smooth, interior wall is facing outwards.
2. Stretch a size 13 O-ring over a 20cc syringe or a size 10 O-ring over a 10cc syringe with an approximately 0.6 cm (0.25 in) hole drilled into one side.
3. Pull the artery onto the syringe, taking care not to tear the artery or have a too loose fit. The artery should fit snugly to the syringe. Slide another O-ring of the same size onto the bottom of the syringe
4. Carefully pull both O-rings over the ends of the artery. The distance between the O-rings should be at least 3.5 cm
5. Using the blade of some surgical scissors, gently scrape the surface of the artery in order to roughen the surface of the artery.
6. Use a 18-gauge needle to poke a hole through the artery over the site of the hole in the

syringe barrel (see note above)

7. The tip of the biopsy punch is inserted through the hole in the artery. Depress the punch's plunger to make an open hole in the artery. Repeat a couple of times to ensure that the hole is open and free of connective tissue.
8. Patch holes left by collateral arteries. Generally this is done by cutting a patch from a latex glove and gluing it over the hole with cyanoacrylate glue. Allow the glue to cure for at least 10 minutes.
9. Place the artery in the warmed, moistened container and place in the incubation chamber. Allow the arteries to warm for at least 30 minutes.

#### **4. Solution and Equipment Preparation**

1. Check to see that the water bath and incubation chamber are maintained at 29-33°C.
2. Make sure that there is sufficient 0.9% saline in the pump's reservoir for completion of the day's assays. Add more if needed.
3. Place 0.9% saline and 0.9% saline with a few drops of red food coloring added into containers in a water bath so that the solutions will be warmed prior to performing the assay.
4. Prepare the container for warming the arteries in the incubation chamber by lining with KimWipes™ and adding a small amount of water to keep the arteries moist.
5. Check the tubing for air bubbles. If bubbles exist, turn on the pump and allow the 0.9% saline to flow until all bubbles are removed.

## 5. Application of the Dressing

1. Open the haemostatic dressing pouch and remove haemostatic dressing
2. Place the haemostatic dressing, mesh backing side UP, over the hole in the artery
3. Slowly wet the haemostatic dressing with an amount of saline appropriate for the article being tested

**NOTE:** A standard (13-15 mg/cm<sup>2</sup> of fibrinogen) 2.4 x 2.4 cm haemostatic dressing should be wet with 800 µl of saline or other blood substitute. The amount of saline used can be adjusted depending on the requirements of the particular experiment being performed; however, any changes should be noted on the data collection forms.

**NOTE:** Wet the haemostatic dressing drop wise with 0.9% saline warmed to 29-33°C or other blood substitute, taking care to keep the saline from running off the edges. Any obvious differences in wetting characteristics from the positive control should be noted on data collection forms.

4. Place the shield gently onto the haemostatic dressing, taking care that it lies flat between the O-rings. Press lightly to secure in place
5. Wrap the artery and haemostatic dressing with plastic wrap
6. Wrap with blood pressure cuff, taking care that the bladder is adjacent to the haemostatic dressing.
7. Pump up the bladder to 100-120 mmHg, and monitor the pressure and pump again if it falls below 100 mmHg. Maintain pressure for 5 minutes.

**NOTE:** Time and pressure can be altered according to the requirements of the experiment; changes from the standard conditions should be noted on the data collection forms.

8. After polymerization, carefully unwrap the artery and note the condition of the haemostatic dressing. Any variation from the positive control should be noted on the data collection form.

**[00100] EXCLUSION CRITERION:** The mesh backing must remain over the hole in the artery. If it has shifted during the polymerization and does not completely cover the hole the haemostatic dressing must be excluded.

### ***Testing Procedure***

#### **1. Diagram of testing equipment set-up**

**[00101]** A diagram of the testing equipment set-up is shown in Figure 2. Some additional components, not shown, may be utilized to read out (pressure gauge) or control the pressure within the system as desired.

#### **1. Equipment and Artery Assembly**

**[00102]** Fill the artery and syringe with red 0.9% saline warmed to 37°C, taking care to minimize the amount of air bubbles within the syringe & artery. Filling the artery with the opening uppermost can assist with this. Attach the artery and syringe to the testing apparatus, making sure that there are as few air bubbles in the tubing as possible. The peristaltic pump should be calibrated so that it delivers approximately 3 ml/min. If available, the PLC should be operated according to a

pre-determined range of pressures and hold times as appropriate for the article being tested. If under manual control, the pressure/time profile to be followed is attained by manually turning the pump on and off while referencing the system pressure as read out by one or more pressure-reading components of the system. Following the conclusion of testing, the haemostatic dressing is subjectively assessed with regard to adhesion to the artery and formation of a plug in the artery hole. Any variations from the positive control should be noted on the data collection form.

### *Success Criteria*

**[00103]** Haemostatic dressings that are able to withstand pressures for 3 minutes are considered to have passed the assay. When a haemostatic dressing has successfully passed the assay the data collection should be stopped immediately so that the natural decrease in pressure that occurs in the artery once the test is ended isn't included on the graphs. Should the operator fail to stop data collection, these points can be deleted from the data file to avoid confusing the natural pressure decay that occurs post-test with an actual dressing failure. The entire testing period from application of the haemostatic dressing to completion must fall within pre-established criteria. The maximum pressure reached should be recorded on the data collection form.

**NOTE:** Typical challenge is 250 mmHg for three minutes in one step, but that may be altered based on the article being tested. Changes from the standard procedure should be noted on the data collection forms.

### *Failure criteria*

**[00104]** Haemostatic dressings that start leaking saline at any point during testing are considered to have failed the assay.

**NOTE:** Build failures that are caused by artery swelling can be ignored and the test continued or re-started (as long as the total testing time doesn't fall beyond the established limit).

**[00105]** When leakage does occur, the pressure should be allowed to fall ~20 mmHg before data collection is stopped so that the failure is easily observed on the graphs. The pressures at which leakage occurred should be recorded on the data collection form. Should the data collection stop in the middle of the experiment due to equipment failure the data can be collected by hand at 5 second intervals until the end of the test or haemostatic dressing failure, whichever happens first. The data points should be recorded on the back of the data collection form, clearly labeled, and entered by hand into the data tables.

### ***Exclusion Criteria***

**[00106]** If the total testing period exceeds the maximum allowed for that procedure, regardless of cause, results must be excluded. If there are leaks from collaterals that can't be fixed either by patching or finger pressure the results must be excluded. If the test fails because of leaks at the O-rings, the results must be excluded. If the mesh backing does not completely cover the hole in the artery, the results must be excluded.

### **Adherence Performance Testing**

#### **1. Equipment and Supplies**

**[00107]** Hemostat(s), Porcine artery and haemostatic dressing (usually after completion of the EVPA Assay although it does not need to be performed to do the Adherence Assay).

- 1. Preparation of the Artery + Dressing**

[00108] After application of the dressing without completion of the EVPA Assay, the dressing is ready for the Adherence Assay and Weight Limit Test (if applicable). After application of the dressing and subsequent EVPA Analysis, the artery and syringe system is then disconnected slowly from the pump so that solution does not spray everywhere. The warmed, red saline solution from the EVPA Assay remains in the syringe until the Adherence Assay and Weight Limit Test (if applicable) is completed.

[00109] ***Performance of the Adherence Assay***

1. After preparation of the artery and dressing (with or without EVPA analysis), gently lift the corner of the mesh and attach a hemostat of known mass to the corner.

**NOTE:** If the FD developed a channel leak during the performance of the EVPA Assay, test the adherence on the opposite of the haemostatic dressing to obtain a more accurate assessment of the overall adherence.

2. Gently let go of the hemostat, taking care not to allow the hemostat to drop or twist. Turn the syringe so that the hemostat is near the top and allow the hemostat to peel back the dressing as far as the dressing will permit. This usually occurs within 10 seconds. After the hemostat has stopped peeling back the dressing, rate the adherence of the bandage according to the following scale:

Dressing Performance Score	Amount of Adherence
4	90+%
3	75-90%
2	50-75%
1	~50%
0.5	Only the plug holds the hemostat
0	No adherence

### *Exclusion Criteria*

[00110] The mesh backing must remain over the hole in the artery. If it has shifted during the polymerization and does not completely cover the hole the haemostatic dressing must be excluded.

### *Success Criteria*

[00111] Dressings that are given an adherence score of 3 are considered to have passed the assay.

### *Failure Criteria*

[00112] If a dressing does not adhere to the artery after application and/or prior to performing the EVPA assay, it is given a score of 0 and fails the adherence test. If a dressing receives a score  $\leq 2$ , the dressing is considered to have failed the Adherence Assay.

### Weight Held Performance Assay

[00113] After the initial scoring of the “Adherence Test”, weights may then be added to the hemostat in an incremental manner until the mesh backing is pulled entirely off of the artery. The maximum weight that the dressing holds is then recorded as a measure of the amount of weight the dressing could hold attached to the artery.

### Moisture Assay

[00114] Moisture determinations were carried out using a Brinkman Metrohm Moisture Analyzer System. The system contains the following individual components, 774 Oven Sample Processor, 774SC Controller, 836 Titrando, 5ml and 50ml 800 Dosino Units and a 801 Stirrer. The system was connected to a computer using the Brinkman Tiamo software for data collection, analysis and storage. The moisture system is set-up and run according to the manufactures recommendations and specifications to measure the moisture content of lyophilized samples using the Karl Fischer method.

[00115] All components were turned on and allowed to reach operating temperature prior to use. Lactose and water were run as standards and to calibrate the instrument. Once the machine was successfully calibrated, samples were prepared as follows. Dressing pieces weighing at least 30mg were placed into vials and capped. The vials were placed in the 774 Oven Sample Processor in numerical order, and one empty capped vial is placed in the conditioning space. The machine was then run to determine the moisture content (residual moisture) in the controls and samples.

## WHAT IS CLAIMED IS:

1. A solid dressing for treating wounded tissue in a mammal comprising at least one haemostatic layer consisting essentially of thrombin and a fibrinogen component, wherein said thrombin is present in an amount between about 0.250 Units/mg of fibrinogen component and 0.062 Units/mg of fibrinogen component.
2. The solid dressing of claim 1, further comprising at least one support layer.
3. The solid dressing of claim 2, wherein said support layer comprises a backing material.
4. The solid dressing of claim 2, wherein said support layer comprises an internal support material.
5. The solid dressing of claim 2, wherein said support layer comprises a resorbable material.
6. The solid dressing of claim 2, wherein said support layer comprises a non-resorbable material.
7. The solid dressing of claim 6, wherein said non-resorbable material is selected from the group consisting of silicone polymers, gauze and latexes.
8. The solid dressing of claim 3, further comprising at least physiologically acceptable adhesive between said haemostatic layer and said backing layer.

9. The solid dressing of claim 5, wherein said resorbable material is selected from the group consisting of proteinaceous materials and carbohydrate substances.
10. The solid dressing of claim 9, wherein said proteinaceous material is at least one substance selected from the group consisting of keratin, silk, fibrin, collagen and gelatin.
11. The solid dressing of claim 9, wherein said carbohydrate substance is selected from the group consisting of alginic acid and salts thereof, chitin, chitosan, hyaluron, hyaluronic acid, cellulose, n-acetyl glucosamine, proteoglycans, glycolic acid polymers, lactic acid polymers, glycolic acid/lactic acid co-polymers and mixtures of two or more thereof.
12. The solid dressing of claim 1, wherein said haemostatic layer also contains a fibrin cross-linker and/or a source of calcium ions.
13. The solid dressing of claim 1, wherein said haemostatic layer also contains one or more of the following: at least one filler, at least one solubilizing agent, at least one foaming agent and at least one release agent.
14. The solid dressing of claim 13, wherein said filler is selected from the group consisting of sucrose, lactose, maltose, keratin, silk, fibrin, collagen, gelatin, albumin, polysorbate, chitin, chitosan, hyaluron, hyaluronic acid, alginic acid and salts thereof, cellulose, proteoglycans, glycolic acid polymers, lactic acid polymers, glycolic acid/lactic acid co-polymers, and mixtures of two or more thereof.

15. The solid dressing of claim 13, wherein said solubilizing agent is selected from the group consisting of sucrose, lactose, maltose, dextrose, mannose, trehalose, mannitol, sorbitol, hyaluron, hyaluronic acid, albumin, sorbate, polysorbate, and mixtures of two or more thereof.
16. The solid dressing of claim 13, wherein said release agent is selected from the group consisting of gelatin, hyaluron, hyalruonic acid, mannitol, sorbitol, polysorbate, sorbitan, lactose, maltose, trehalose, sorbate, glucose and mixtures of two or more thereof.
17. The solid dressing of claim 13, wherein said foaming agent is selected from the group consisting of mixtures of sodium bicarbonate/citric acid, sodium bicarbonate/acetic acid, calcium carbonate/citric acid and calcium carbonate/acetic acid.
18. The solid dressing of claim 1, wherein said haemostatic layer also contains at least one therapeutic supplement selected from the group consisting of antibiotics, anticoagulants, steroids, cardiovascular drugs, growth factors, antibodies (poly and mono), chemoattractants, anesthetics, antiproliferatives/antitumor agents, antivirals, cytokines, colony stimulating factors, antifungals, antiparasitics, antiinflammatories, antiseptics, hormones, vitamins, glycoproteins, fibronectin, peptides, proteins, carbohydrates, proteoglycans, antiangiogenins, antigens, nucleotides, lipids, liposomes, fibrinolysis inhibitors and gene therapy reagents.
19. The solid dressing of claim 1, wherein said haemostatic layer is made from a single aqueous solution containing a mixture of said fibrinogen component and said thrombin.

20. The solid dressing of claim 1, wherein said haemostatic layer is cast as a single piece.
21. The solid dressing of claim 1, wherein said haemostatic layer is substantially homogeneous throughout.
22. The solid dressing of claim 1, wherein said haemostatic layer is composed of a plurality of particles, each of said particles consisting essentially of fibrinogen and thrombin.
23. The solid dressing of claim 22, wherein said haemostatic layer further contains at least one binding agent in an amount effective to improve the adherence of said particles to one another.
24. The solid dressing of claim 25, wherein said binding agent is selected from the group consisting of sucrose, mannitol, sorbitol, gelatin, maltose, povidone, hyaluron, hyaluronic acid, chitosan and carboxymethylcellulose..
25. The solid dressing of claim 1, wherein said haemostatic layer is a monolith.
26. The solid dressing of claim 1, wherein said haemostatic layer has been lyophilized.
27. The solid dressing of claim 1, wherein said haemostatic layer has moisture content of at least 6%.
28. The solid dressing of claim 1, wherein said haemostatic layer has moisture content of less than 6%.

29. The solid dressing of claim 1, wherein said fibrinogen component is a mammalian fibrinogen.
30. The solid dressing of claim 29, wherein said mammalian fibrinogen is selected from the group consisting of bovine fibrinogen, porcine fibrinogen, ovine fibrinogen, equine fibrinogen,, caprine fibrinogen, feline fibrinogen, canine fibrinogen, murine fibrinogen and human fibrinogen.
31. The solid dressing of claim 1, wherein said fibrinogen component is selected from the group consisting of bird fibrinogen and fish fibrinogen.
32. The solid dressing of claim 1, wherein said fibrinogen component is selected from the group consisting of human fibrinogen, human fibrin I, human fibrin II, human fibrinogen  $\alpha$  chain, human fibrinogen  $\beta$  chain, human fibrinogen  $\gamma$  chain, and mixtures of two or more thereof.
33. The solid dressing of claim 29, 31 or 32, wherein said fibrinogen is selected from the group consisting of recombinantly produced fibrinogen and transgenic fibrinogen.
34. The solid dressing of claim 29, wherein said mammalian fibrinogen is present in an amount between 1.5 mg/cm<sup>2</sup> of the wound-facing surface of said dressing and 13.0 mg/cm<sup>2</sup> of the wound-facing surface of said dressing.
35. The solid dressing of claim 1, wherein said thrombin is mammalian thrombin.

36. The solid dressing of claim 35, wherein said mammalian thrombin is selected from the group consisting of bovine thrombin, porcine thrombin, ovine thrombin, equine thrombin, caprine thrombin, feline thrombin, canine thrombin, murine thrombin and human thrombin.
37. The solid dressing of claim 1, wherein said thrombin is selected from the group consisting of bird thrombin and fish thrombin.
38. The solid dressing of claim 35 or 37, wherein said thrombin is selected from the group consisting of recombinantly produced thrombin and transgenic thrombin.
39. The solid dressing of claim 19, wherein said therapeutic supplement is present in an amount equal to or greater than its solubility limit in fibrin.
40. A method of treating wounded tissue in a mammal, comprising placing a solid dressing of claim 1 to said wounded tissue and applying sufficient pressure to said dressing for a sufficient time for enough fibrin to form to reduce the loss of blood and/or other fluid from said wounded tissue.

FIGURE 1A.

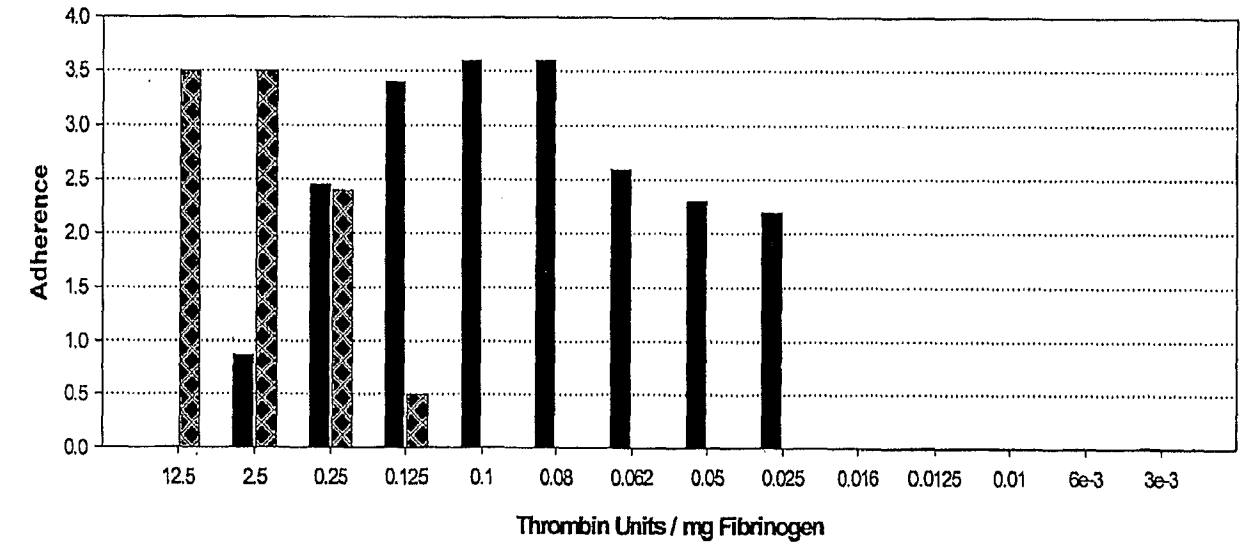
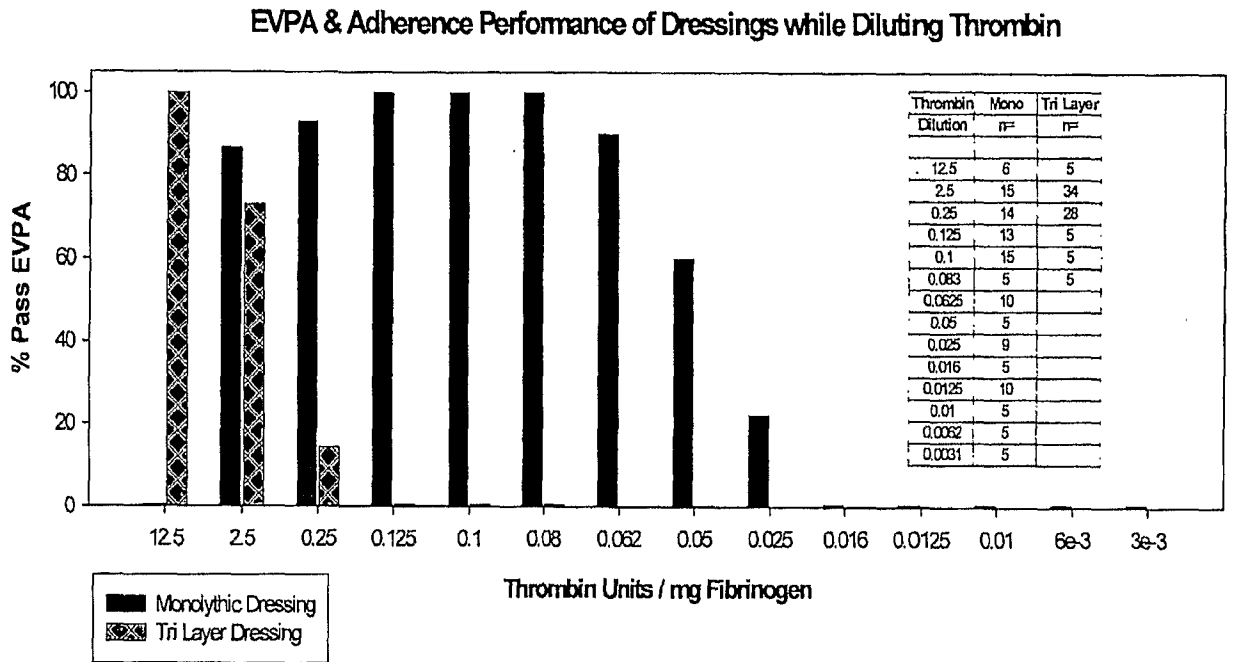


FIGURE 1B.

FIGURE 2.

