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(54) Title: HEMOSTATIC SPONGE

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The present invention provides a hemostatic composite sponge comprising a porous matrix of a biomaterial and a material enhancing the adherence of said sponge to the applied tissue stably associated with at least one surface of said sponge, a method of producing these sponges and their use in hemostasis.

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(54) **Title**: HEMOSTATIC SPONGE

(57) **Abstract**: The present invention provides a hemostatic composite sponge comprising a porous matrix of a biomaterial and a material enhancing the adherence of said sponge to the applied tissue stably associated with at least one surface of said sponge, a method of producing these sponges and their use in hemostasis.

Hemostatic sponge

Field of the invention

The present invention relates to the field of hemostatic sponges, a method of producing said sponges and their uses in hemostasis.

Background of the invention

Biological glues based on coagulation factors of human or animal origin have long been known. A method for producing tissue adhesives based on fibrinogen and factor XIII has been described in US 4,362,567, US 4,298,598 and US 4,377,572. The tissue adhesives are usually applied together with a separate component containing thrombin, which is enzymatically acting on fibrinogen to form fibrin, and on factor XIII to form the active factor XIIIa, which cross-links the fibrin to obtain a stable fibrin clot.

Collagen pads have been used for many years to improve wound healing or to stop bleeding. Their mechanism of action in hemostasis is based on platelet aggregation and activation, the formation of thrombin on the surface of activated platelets and the formation of a hemostatic fibrin clot by the catalytic action of thrombin on fibrinogen. To improve the hemostatic action of collagen pads or sheets it has been suggested to include factors of hemostasis within such pads.

In US 4,600,574 a tissue adhesive based on collagen combined with fibrinogen and factor XIII is described. This material is provided in the lyophilized form, ready for use. The fibrinogen and factor XIII are combined with the collagen by impregnating the collagenous flat material with a solution comprising fibrinogen and factor XIII, and lyophilizing said material.

The WO 97/37694 discloses a hemostatic sponge based on collagen and an activator or proactivator of blood coagulation homogeneously distributed therein. This sponge is provided in a dry form, which could be air-dried or lyophilized. However, it still contains a water content of at least 2%.

US 5,614,587 discusses bioadhesive compositions comprising cross-linked collagen using a multifunctionally activated synthetic hydrophilic polymer, as well as methods of using such compositions to effect adhesion between a first surface and a second surface, wherein at least one of the first and second surfaces can be a native tissue surface.

Collagen-containing compositions which have been mechanically disrupted to alter their physical properties are described in US 5,428,024, US 5,352,715, and US 5,204,382. These patents generally relate to fibrillar and insoluble collagens. An injectable collagen composition is described in US 4,803,075. An injectable bone/cartilage composition is described in US 5,516,532. A collagen-based delivery matrix comprising dry particles in the size range from 5 µm to 850 µm which may be suspended in water and which has a particular surface charge

density is described in WO 96/39159. A collagen preparation having a particle size from 1 µm to 50 µm useful as an aerosol spray to form a wound dressing is described in US 5,196,185. Other patents describing collagen compositions include US 5,672,336 and US 5,356,614.

Summary of the invention

The subject of the invention is a hemostatic porous composite sponge comprising a matrix of a biomaterial and a material enhancing the adherence of said sponge to the applied tissue stably associated with at least one surface of said sponge, wherein said material is essentially free of a hydrogel forming component.

It has been found that previous pads of fibrous biomaterials, in particular collagen pads, for wound healing failed to induce hemostasis at conditions with impaired hemostasis (e.g. after heparinization). The inventive sponge improves hemostasis.

It has further been found that if a further material is present on a surface of the biomatrix material as an active hemostatic layer such a layer tends to be instable in that the material has a tendency to detach from the sponge, especially during application of the sponge on the tissue and when being adjusted to the geometry of said tissue.

It has also been found that the absence of a further hydrogel forming component, such as e.g. a particulate material, e.g. gelatin particles, has advantageous properties especially with regards to lower swelling properties of the sponge as a whole.

It has been possible to overcome these drawbacks in that a sponge of the present invention is provided.

A further aspect relates to a method of manufacturing a hemostatic porous sponge comprising

a) providing a porous sponge of a matrix of a biomaterial,

b) providing a material enhancing the adherence of said sponge to the applied tissue in the form of a suspension, a solution or powder, wherein said material is essentially free of a hydrogel forming component,

c) contacting a) and b) so that the material of b) is stably associated with at least one surface of said sponge so that a hemostatic composite sponge is obtained, optionally

d) drying the composite sponge obtained in step c), optionally

e) sterilizing said composite sponge obtained in step c) or d).

Another aspect relates to a method of treating an injury comprising administering a he-

mostatic porous composite sponge to the site of injury.

Also provided is a kit for preparing a wound coverage, comprising a sponge as herein disclosed and pharmaceutically active substances. This kit and its components are in particular for the manufacture of a medical sponge for the treatment of an injury.

In accordance with another aspect, there is provided a hemostatic composite sponge comprising a porous sponge of a matrix of a biomaterial and an adherence-enhancing material enhancing the adherence of said sponge to an applied tissue stably associated with at least one surface of said sponge, wherein said adherence-enhancing material is a mixture of two pre-polymers comprising a first cross-linkable component which includes multiple nucleophilic groups, and a second cross-linkable component which includes multiple electrophilic groups and which cross-links with the first cross-linkable component upon contact with a biological fluid and said adherence-enhancing material is essentially free of a hydrogel forming component.

In accordance with an aspect, there is provided a hemostatic composite sponge comprising a porous sponge of a matrix of a biomaterial and an adherence-enhancing material enhancing the adherence of said sponge to an applied tissue stably associated with at least one surface of said sponge, wherein said adherence-enhancing material is a mixture of two pre-polymers comprising a first cross-linkable component which includes multiple nucleophilic groups, and a second cross-linkable component which includes multiple electrophilic groups and which cross-links with the first cross-linkable component upon contact with a biological fluid and said adherence-enhancing material is essentially free of a hydrogel forming component, wherein the hemostatic sponge is for use in a laparoscopic application.

In accordance with an aspect, there is provided a method of manufacturing a hemostatic composite sponge comprising

a) providing a porous sponge of a matrix of a biomaterial,

b) providing an adherence-enhancing material to an applied tissue in the form of a suspension, a solution or powder, said adherence-enhancing material enhancing the adherence of said sponge to an applied tissue stably associated with at least one surface of said sponge, wherein said adherence-enhancing material is a mixture of two pre-polymers comprising a first cross-linkable component which includes multiple nucleophilic groups, and a second cross-linkable component which includes multiple electrophilic groups and which cross-links with the first cross-linkable component upon contact with a biological fluid and said adherence-enhancing material is essentially free of a hydrogel forming component,

c) contacting a) and b) so that the adherence-enhancing material of b) is stably associated with at least one surface of said sponge so that the hemostatic composite sponge is obtained, optionally

d) drying the composite sponge obtained in step c), optionally,

e) sterilizing said composite sponge obtained in step c) or d).

In accordance with an aspect, there is provided a use of a hemostatic composite sponge for the treatment of one or more of a wound, a hemorrhage, a damaged tissue and a bleeding

tissue, the hemostatic composite sponge comprising a porous sponge of a matrix of a biomaterial and an adherence-enhancing material enhancing the adherence of said sponge to an applied tissue stably associated with at least one surface of said sponge, wherein said adherence-enhancing material is a mixture of two pre-polymers comprising a first cross-linkable component which includes multiple nucleophilic groups, and a second cross-linkable component which includes multiple electrophilic groups and which cross-links with the first cross-linkable component upon contact with a biological fluid and said adherence-enhancing material is essentially free of a hydrogel forming component.

In aspects, the adherence-enhancing material is present in a concentration of 5 to 500 mg per cm^2 of biomaterial.

Those skilled in the art will readily understand that all preferred embodiments disclosed in the following are examples of specific embodiments, but are not necessarily limiting the general inventive concept. Furthermore, all special embodiments can be read on all inventive aspects and embodiments in any combination, if not mutually exclusive. All equivalents or obvious alterations or modifications as recognized by those skilled in the art are included by the present invention.

Detailed description of the invention

The object of an aspect of the invention is a hemostatic porous composite sponge comprising a matrix of a biomaterial and a material enhancing the adherence of said sponge to the applied tissue stably associated with at least one surface of said sponge, wherein said material is essentially free of a hydrogel forming component.

"Stably associated" according to the present invention means that the material enhancing the adherence of said sponge to the applied tissue stays firmly associated with the sponge during application of this sponge to the tissue and adjusting to the geometry of said tissue, even if the sponge is e.g. bended during that application.

Preferably the biomaterial is collagen, a protein, a biopolymer, or a polysaccharide. Especially preferred is a biomaterial selected from the group consisting of collagen, gelatin, fibrin, a polysaccharide, e.g. chitosan, and a derivative thereof, more preferred collagen and chitosan, especially preferred collagen.

The sponge is a porous network of a biomaterial able to absorb body fluids when applied to the site of an injury. Furthermore, the sponge is usually flexible and suitable to be applied on diverse tissues and locations with various shapes.

The collagen used for the present invention can be from any collagen suitable to form a gel, including a material from liquid, pasty, fibrous or powdery collageneous materials that can be processed to a porous or fibrous matrix. The preparation of a collagen gel for the production of a sponge is e.g. described in the EP 0891193 and may include acidification until gel formation occurs and subsequent pH neutralisation. To improve gel forming capabilities or solubili-

ty the collagen may be (partially) hydrolyzed or modified, as long as the property to form a stable sponge when dried is not diminished.

The collagen sponge according to the present invention preferably has a lower density

as compared to the density of a collagen film. Preferably the density is between about 5 to about 100 mg per cm^3, whereas densities of films are higher than about 650 mg per cm^3. An especially preferred collagen sponge according to the present invention is the one marketed under the name Matristypt®.

The collagen or gelatin of the sponge matrix is preferably of animal origin, preferably bovine or equine. However, also human collagen might be used in case of a hypersensitivity of the patient towards xenogenic proteins. The further components of the sponge are preferably of human origin, which makes the sponge suitable especially for the application to a human.

In a preferred embodiment the matrix material of the fibrous biocompatible polymer which forms the porous network of the sponge constitutes of between 1-50%, 1-10%, preferably about 3% of the dried porous sponge (w/w-%).

In a preferred embodiment the material enhancing the adherence of said sponge to the applied tissue, in the following called "the material", is a mixture of two pre-polymers comprising a first cross-linkable component and a second cross-linkable component that cross-links with the first cross-linkable component under reaction enabling conditions or a formed polymer in association with said sponge.

The material enhancing the adherence of said sponge to the applied tissue stably associated with at least one surface of said sponge is essentially free of a hydrogel forming component, especially free of a particulate hydrogel forming component, e.g. gelatin particulate material or gelatin particles.

More preferably said first and/or second cross-linkable component comprise a derivative of polyethylene glycol (PEG), e.g. a derivative which is able to react under given conditions. Preferably one of the cross-linkable components is capable of covalently reacting with tissue.

Such materials suitable for a sponge for use as a hemostat are e.g. disclosed in the WO2008/016983 and commercially available under the trademark CoSeal®. Preferred materials mediate adjunctive hemostasis by themselves, and can be suitable to mechanically seal areas of leakage. Such materials are for example bioresorbable polymers, in particular polymers that cross-link and solidify upon exposure to body fluids. In further embodiments the material is resorbable and/or biocompatible and can be degraded by a subject, in particular a human subject, in less than 6 months, less than 3 months, less than 1 month or less than 2 weeks.

A special material enhancing the adherence of said sponge to the applied tissue may comprise a first cross-linkable component, a second cross-linkable component that cross-links with the first cross-linkable component under reaction enabling conditions, wherein the first and second cross-linkable component cross-link to form a layer.

The first cross-linkable component can include multiple nucleophilic groups and the se-

cond cross-linkable component can include multiple electrophilic groups. Upon contact with a biological fluid, or in other reaction enabling conditions, the cross-linkable first and second components cross-link to form a porous matrix having interstices.

In some aspects, the first cross-linkable component of the material includes a multi-nucleophilic polyalkylene oxide having m nucleophilic groups, and the second cross-linkable component includes a multi-electrophilic polyalkylene oxide. The multi-nucleophilic polyalkylene oxide can include two or more nucleophilic groups, for example NH_2, -SH, -H, $-PH_2$, and/or -CO-NH-NH_2. In some cases, the multi-nucleophilic polyalkylene oxide includes two or more primary amino groups. In some cases, the multi-nucleophilic polyalkylene oxide includes two or more thiol groups. The multi-nucleophilic polyalkylene oxide can be polyethylene glycol or a derivative thereof. In some cases, the polyethylene glycol includes two or more nucleophilic groups, which may include a primary amino group and/or a thiol group. The multi-electrophilic polyalkylene oxide can include two or more electrophilic groups such as $CO_2N(COCH_2)_2$, -CO_2H, -CHO, -$CHOCH_2$, -N=C=O, -$SO_2CH=CH_2$, $N(COCH)_2$, and/or -S-S-(C_5H_4N). The multi-electrophilic polyalkylene oxide may include two or more succinimidyl groups. The multi-electrophilic polyalkylene oxide may include two or more maleimidyl groups. In some cases, the multi-electrophilic polyalkylene oxide can be a polyethylene glycol or a derivative thereof.

In special embodiments the first and/or second cross-linkable component is/are synthetic polymers, preferably comprising PEG. The polymer can be a derivative of PEG comprising active side groups suitable for cross-linking and adherence to a tissue. Preferably, the adhesive comprises succinimidyl, maleimidyl and/or thiol groups. In a two polymer set-up, one polymer may have succinyl or maleimidyl groups and a second polymer may have thiol or amino groups which can attach to the groups of the first polymer. These or additional groups of the adhesive may facilitate the adherence to a tissue.

Preferably the material enhancing the adherence of said sponge to the applied tissue, such as modified PEG materials as mentioned before, is present in a range of 5 to 50 mg/cm^2 of the biomaterial, preferably 10 to 20 mg/cm^2 of the biomaterial, e.g. collagen.

The sponge as a whole is biodegradable, being suitable for biological decomposition in vivo, or bioresorbable, i.e. able to be resorbed in vivo. Full resorption means that no significant extracellular fragments remain. A biodegradable material differs from a non-biodegradable material in that a biodegradable material can be biologically decomposed into units which may either be removed from the biological system and/or chemically incorporated into the biological system. In a preferred embodiment the particular material, the matrix material or sponge as a whole can be degraded by a subject, in particular a human subject, in less than 6 month, less than 3 month, less than 1 month, less than 2 weeks.

In a preferred embodiment the sponge has the material enhancing the adherence of said sponge to the applied tissue in the form of a continuous or discontinuous layer on at least one surface of said sponge.

The sponge of the present invention preferably has an overall thickness of less than 2.5 mm, more preferred about 1 mm to about 2.5 mm.

The sponge of the present invention is preferably used in minimal invasive surgery, e.g. for laparoscopic application.

The sponge may be dried and after drying, the sponge may have a water content of at least 0.5 (percentages given in w/w here). In certain embodiments the sponge can be freeze-dried or air-dried.

The sponge may further comprise an activator or proactivator of blood coagulation, including fibrinogen, thrombin or a thrombin precursor, as e.g. disclosed in US 5,714,370. Thrombin or the precursor of thrombin is understood as a protein that has thrombin activity and that induces thrombin activity when it is contacted with blood or after application to the patient, respectively. Its activity is expressed as thrombin activity (NIH-Unit) or thrombin equivalent activity developing the corresponding NIH-Unit. The activity in the sponge can be 100 – 10.000, preferably 500 - 5.000. In the following thrombin activity is understood to comprise both, the activity of thrombin or any equivalent activity. A protein with thrombin activity might be selected from the group consisting of alpha-thrombin, meizothrombin, a thrombin derivative or a recombinant thrombin. A suitable precursor is possibly selected from the group consisting of: prothrombin, factor Xa optionally together with phospholipids, factor IXa, activated prothrombin complex, FEIBA, any activator or a proactivator of the intrinsic or extrinsic coagulation, or mixtures thereof.

The hemostatic sponge according to the invention might be used together with further physiologic substances. For example, the sponge preferably further comprises pharmacologically active substances, among them antifibrinolytics, such as a plasminogenactivator-inhibitor or a plasmin inhibitor or an inactivator of fibrinolytics. A preferred antifibrinolytic is selected from the group consisting of aprotinin or an aprotinin derivative, alpha2-macroglobulin, an inhibitor or inactivator of protein C or activated protein C, a substrate mimic binding to plasmin that acts competitively with natural substrates, and an antibody inhibiting fibrinolytic activity.

As a further pharmacologically active substance an antibiotic, such as an antibacterial or antimycotic might be used together with the sponge according to the invention, preferably as a component homogeneously distributed in the sponge. Further bioactive substances such as growth factors and/or pain killers may be also present in the inventive sponge. Such a sponge might be useful in e.g. wound healing.

Further combinations are preferred with specific enzymes or enzyme inhibitors, which may regulate, i.e. accelerate or inhibit, the resorption of the sponge. Among those are collagenase, its enhancers or inhibitors. Also, a suitable preservative may be used together with the sponge or may be contained in the sponge.

Although a preferred embodiment relates to the use of the hemostatic sponge which contains the activator or proactivator of blood coagulation as the only active component, further

substances that influence the velocity of blood coagulation, hemostasis and quality of the sealing, such as tensile strength, inner (adhesive) strength and durability might be comprised.

Procoagulants that enhance or improve the intrinsic or extrinsic coagulation, such as factors or cofactors of blood coagulation, factor XIII, tissue factor, prothrombin complex, activated prothrombin complex, or parts of the complexes, a prothrombinase complex, phospholipids and calcium ions, might be used. In case of a surgical procedure where a precise sealing is needed, it might be preferable to prolong the working period after the hemostatic sponge is applied to the patient and before clotting is affected. The prolongation of the clotting reaction will be ensured, if the sponge according to the invention further comprises inhibitors of blood coagulation in appropriate amounts. Inhibitors, such as antithrombin III optionally together with heparin, or any other serine protease inhibitor, are preferred.

It is also preferred to have such additives, in particular the thrombin or a precursor of thrombin evenly distributed in the material in order to prevent local instability or hypercoagulability of the material. Even with a certain water content the thrombin activity is surprisingly stable, probably because of the intimate contact of thrombin and collagen in the homogeneous mixture. Nevertheless, thrombin stabilizers preferably selected from the group consisting of a polyol, a polysaccharide, a polyalkylene glycol, amino acids or mixtures thereof might be used according to the invention. The exemplary use of sorbitol, glycerol, polyethylene glycol, polypropylene glycol, mono- or disaccharides such as glucose or saccharose or any sugar or sulfonated amino acid capable of stabilizing thrombin activity is preferred.

In another embodiment a biocompatible, resorbable hydrogel capable of absorbing liquid is comtained within the sponge of the present invention.

The present invention also provides a wound coverage comprising a sponge according to the invention. The sponge and all additional layers can be provided in a ready to use wound coverage in suitable dimensions. The sponge and/or the coverage can be a pad or a sheet, preferably having a thickness of at least 3mm or at least 5mm and/or up to 20mm, depending on the indication. When the relatively thick flexible sponge is applied to a wound it is important that blood and fibrinogen can be absorbed throughout the sponge before fibrin is formed that might act as a barrier for the absorption of further wound secret.

Another aspect of the invention relates to a method of manufacturing a hemostatic porous sponge comprising

a) providing a sponge comprising a matrix of a biomaterial,

b) providing a material enhancing the adherence of said sponge to the applied tissue in the form of a suspension, a solution or powder,

c) contacting a) and b) so that the material of b) is present on at least one surface of said sponge, and optionally

d) drying the sponge obtained in step c).

Drying may include freeze drying or air drying and comprises removing volatile components of the fluid.

In a further aspect the present invention provides a hemostatic porous sponge obtainable by the method according to the invention described above. All preferred embodiments mentioned above for a hemostatic sponge can also be read to this obtainable sponge.

The present invention also provides a method of treating an injury comprising administering a hemostatic porous composite sponge comprising a matrix of a biomaterial and a material enhancing the adherence of said sponge to the applied tissue. The injury may comprise a wound, a hemorrhage, damaged tissue and/or bleeding tissue.

Description of the Figures:

Figures 1 to 4 show hemostatic performances of the sponges prepared according to examples 1 (= Figure 1), 4 (= Figure 2), 5 (= Figure 3) and 6 (= Figure 4) in an animal model as described in Example 10.

The present invention is further exemplified by the following examples without being limited thereto.

The following abbreviations are used:

COH102	Pentaerythritolpoly(ethyleneglycol)ether tetrasuccinimidyl glutarate
COH206	Pentaerythritolpoly(ethyleneglycol)ether tetra-thiol
EtOH	ethanol
PEG	polyethylene glycol
PET	polyethylene terephthalate

EXAMPLES

Example 1: Collagen sponges treated with acidic solution of two reactive PEGs

Aqueous, acidic solutions (pH 3.0, HCl) of COH102 and COH206 with PEG-concentrations (COH102 and COH206 1:1) of 10mg/cm³, 35mg/cm³, 70mg/cm³ and 100mg/cm³ are prepared and filled into 9x7 cm PET-trays. Commercial available bovine collagen sponges (*Matristypt®)*, 9x7 cm, with the same volume as the previously filled PEG-solution are placed on the top of the solutions. After absorption of the PEG-solution, the collagen materials are lyophilized.
After lyophilization the dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be further gamma-sterilized, e.g. with 25kGray.

Example 2: Collagen sponges treated with EtOH-solution of two reactive PEGs

COH102 and COH206 are dissolved in completely dried EtOH. PEG-concentrations (COH102 and COH206 1:1) of 10mg/cm³, 35mg/cm³, 70mg/cm³ and 100mg/cm³ are prepared and the solutions are filled into 9x7 cm PET-trays. Commercial available bovine collagen sponges (*Matristypt®)*, 9x7 cm, with the same volume as the previously filled PEG-solution are placed on the top of the solutions. After absorption of the PEG-solution the collagen materials are dried in a vacuum chamber.
Dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be gamma-sterilized, e.g. with 25kGray.

Example 3: Preparation of collagen-/reactive PEG constructs

22ml of aqueous, acidic solutions (pH 3.0, HCl) containing various concentrations (2.15mg/cm³, 4.3mg/cm³ and 7.2mg/cm³) of bovine corium collagen and PEG (COH102 and COH206 1:1)-concentrations of 7.2 mg/cm³, 14.3mg/cm³, 28.6mg/cm³and 57.3mg/cm³ are prepared, filled into PET-trays and lyophilized.
The dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be gamma-sterilized, e.g. with 25kGray.

Example 4: Preparation of two layer collagen-/reactive PEG constructs

11ml and 22ml of acidic collagen-/PEG-solutions (pH 3.0, HCl) as described in example 3 are filled into PET-trays and immediately frozen at -20°C. On the top of the ice phase 11ml or 22ml of a 1% bovine corium collagen solution, pH 3.0 (HCl) are applied and the constructs obtained are freeze-dried.
The dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be gamma-sterilized, e.g. with 25kGray.

Example 5: Homogeneous coating of collagen sponges with reactive PEGs

A 1:1 powder mixture of COH102 and COH206 is homogeneously distributed onto one surface

of a commercially available collagen sponge or on a sponge prepared after one of the methods as described in example 1, 2, 3 and 4. PEG-amounts of 2mg/cm², 7mg/cm², 10mg/cm², 14mg/cm² and 20mg/cm² are used for the coating. The PEG-powder mixture is fixed on the surface of the sponge, e.g. by melting, such as by placing the sponges with the PEG-powder mixture into a preheated oven at 60 to 65°C for 4 minutes.
The dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be gamma-sterilized, e.g. with 25kGray.

Example 6: Discontinuous coating of collagen sponges with reactive PEGs
Pads are prepared as described in example 5 with the exception that before coating a grid is placed onto the surface of the collagen sponge, so that the surface of the pad is partially shielded and partially not covered by the PEG powder. Grid matrices with a mesh size of 5mm and 10mm are used and removed after powder distribution. Fixation of the powder, packaging and sterilization are those as described in example 5.
These prototypes allow a better penetration of the blood into the collagen pad, where coagulation occurs due to the procoagulant activity of collagen. The reactive PEGs assure the adhesion of the pad to the wound surface.

Example 7: Preparation of constructs of collagen with cross-linked PEG
a) Onto a bovine collagen sponge the reactive PEGs COH102 and COH206 (1:1) are sprayed with a commercial available spray applicator composed of a double syringe and a gas driven spray head (Duplospray™, Baxter). One syringe contains COH102 and COH206 at pH 3.0 and the second syringe buffer, pH 9.4. The polymerization of the two PEG-components occurrs on the surface of collagen immediately after deposition. The sponge may be dried in a vacuum chamber.
b) A collagen sponge is treated with an acidic PEG-solution as described in example 1. In order to start the cross-linking between the two PEG-components and the collagen matrix, the wet sponge is treated with a basic buffer system and may be lyophilized afterwards.

Example 8: Continuous coating of a chitosan-/gelatin sponge with reactive PEG's
A 1:1 powder mixture of COH102 and COH206 is homogeneously distributed onto one surface of a commercially available chitosan-/gelatin (Chitoskin®, Beese Medical) sponge. A PEG-amount of 14mg/cm² is used for the coating. The PEG-powder mixture is fixed on the surface of the sponge, e.g. by melting, such as by placing the sponges with the PEG-powder mixture into a preheated oven at 60 to 65°C for 4 minutes.
The dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be gamma-sterilized, e.g. with 25kGray.

Example 9: Coating of a oxidized cellulose fabric with reactive PEG's

A 1:1 powder mixture of COH102 and COH206 is homogeneously distributed onto one surface of a commercially available oxidized cellulose fabric (Traumstem®, Bioster). A PEG-amount of 14mg/cm² is used for the coating. The PEG-powder mixture is fixed on the surface of the sponge, e.g. by melting, such as by placing the sponges with the PEG-powder mixture into a preheated oven at 60 to 65°C for 4 minutes.
The dried sponges may be packed together with desiccants in water vapor impermeable pouches and may be gamma-sterilized, e.g. with 25kGray.

Example 10: Preclinical applications

A sponge as prepared according to the examples is tested in heparinized pigs (1.5-fold ACT) in a liver abrasion model. With a rotating grinding machine a circular bleeding wound with a diameter of 1.8 cm is created on the surface of a liver lobe. A 3x3 cm sponge is applied and moderately pressed against the wound for 2 minutes with a piece of gauze soaked with saline buffer. After removal of the gauze a good hemostatic performance is achieved as shown in Figures 1 to 4.

We claim:

1. A hemostatic composite sponge comprising a porous sponge of a matrix of a biomaterial and an adherence-enhancing material enhancing the adherence of said sponge to an applied tissue stably associated with at least one surface of said sponge, wherein said adherence-enhancing material is a mixture of two pre-polymers comprising a first cross-linkable component which includes multiple nucleophilic groups, and a second cross-linkable component which includes multiple electrophilic groups and which cross-links with the first cross-linkable component upon contact with a biological fluid and said adherence-enhancing material is essentially free of a hydrogel forming component, wherein the hemostatic sponge is for use in a laparoscopic application.

2. The sponge according to claim 1, wherein said biomaterial is selected from the group consisting of collagen, gelatin, fibrin, a polysaccharide, and a derivative thereof.

3. The sponge according to claim 1, wherein said biomaterial is selected from chitosan or a derivative thereof.

4. The sponge according to any one of claims 1 to 3, wherein said first cross-linkable component includes a multi-nucleophilic polyalkylene oxide and wherein said second cross-linkable component includes a multi-electrophilic polyalkylene oxide.

5. The sponge according to any one of claims 1 to 4, wherein said first and/or second cross-linkable component comprise a derivative of polyethylene glycol.

6. The sponge according to any one of claims 1 to 5, wherein said adherence-enhancing material to the applied tissue forms a continuous or discontinuous layer on at least one surface of said sponge.

7. The sponge according to any one of claims 1 to 6 having an overall thickness of about 1 mm to about 2.5 mm.

8. The sponge according to any one of claims 1 to 7 wherein the adherence-enhancing material is present in a concentration of 5 to 500 mg per cm^2 of biomaterial.

9. The sponge according to claim 8 wherein the adherence-enhancing material is present in a concentration between 5 to 100 mg per cm^2 of biomaterial.

10. A method of manufacturing a hemostatic composite sponge comprising

a) providing a porous sponge of a matrix of a biomaterial,

b) providing an adherence-enhancing material to an applied tissue in the form of a suspension, a solution or powder, said adherence-enhancing material enhancing the adherence of said sponge to an applied tissue stably associated with at least one surface of said sponge, wherein said adherence-enhancing material is a mixture of two pre-polymers comprising a first cross-linkable component which includes multiple nucleophilic groups, and a second cross-linkable component which includes multiple electrophilic groups and which cross-links with the first cross-linkable component upon contact with a biological fluid and said adherence-enhancing material is essentially free of a hydrogel forming component,

c) contacting a) and b) so that the adherence-enhancing material of b) is stably associated with at least one surface of said sponge so that the hemostatic composite sponge is obtained, optionally

d) drying the composite sponge obtained in step c), optionally,

e) sterilizing said composite sponge obtained in step c) or d).

11. The method according to claim 10, wherein said biomaterial is selected from the group consisting of collagen, gelatin, fibrin, a polysaccharide, and a derivative thereof.

12. The method according to claim 10, wherein said biomaterial is selected from chitosan or a derivative thereof.

13. The method according to any one of claims 10 to 12, wherein said first cross-linkable component includes a multi-nucleophilic polyalkylene oxide and wherein said second cross-linkable component includes a multi-electrophilic polyalkylene oxide.

14. The method according to any one of claims 10 to 13, wherein said first and/or second cross-linkable component comprise a derivative of polyethylene glycol.

15. The method according to any one of claims 10 to 14, wherein said adherence-enhancing material to the applied tissue forms a continuous or discontinuous layer on at least one surface of said sponge.

16. The method according to any one of claims 10 to 15 having an overall thickness of about 1 mm to about 2.5 mm.

Figure 1

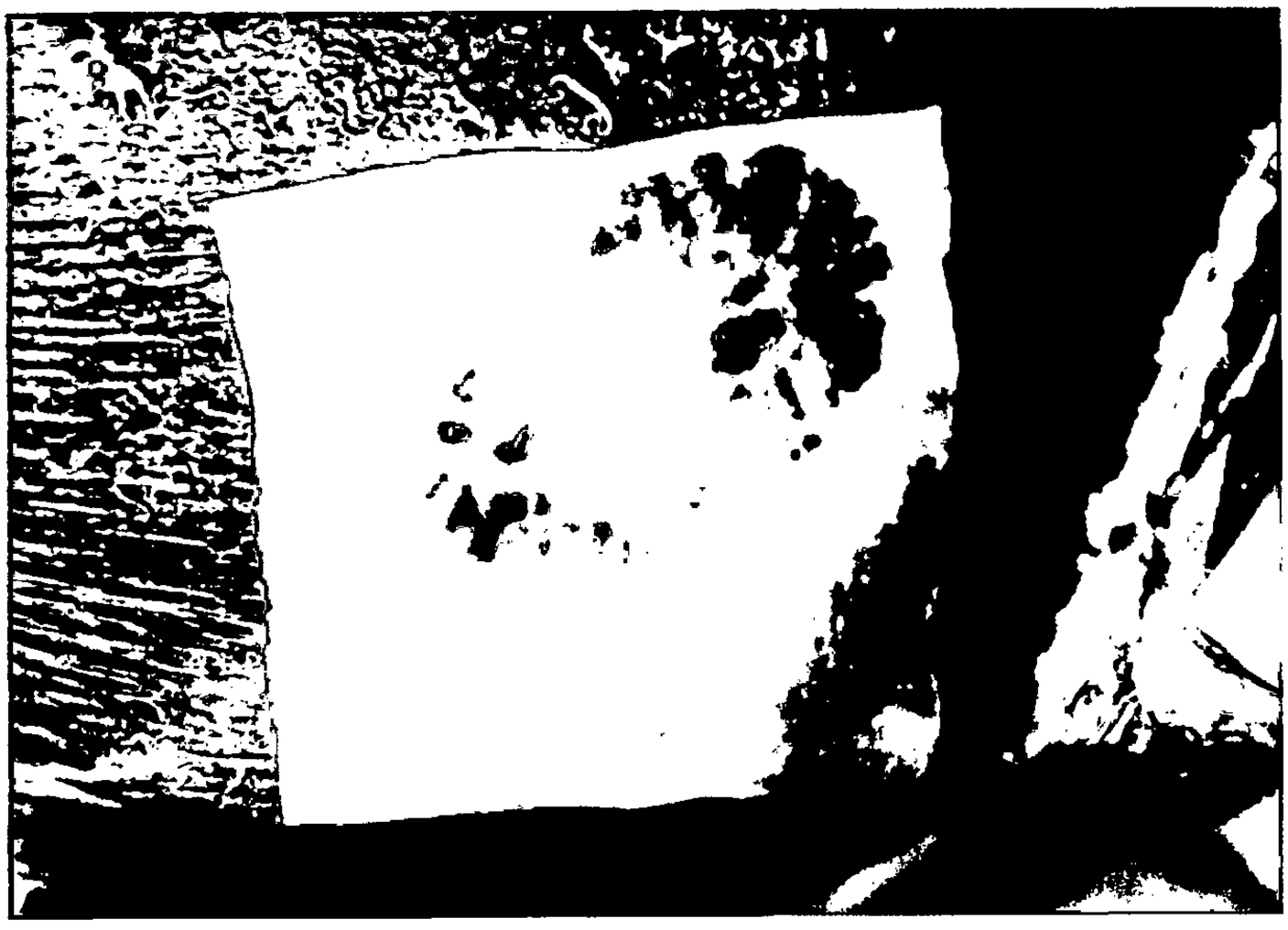

Figure 2

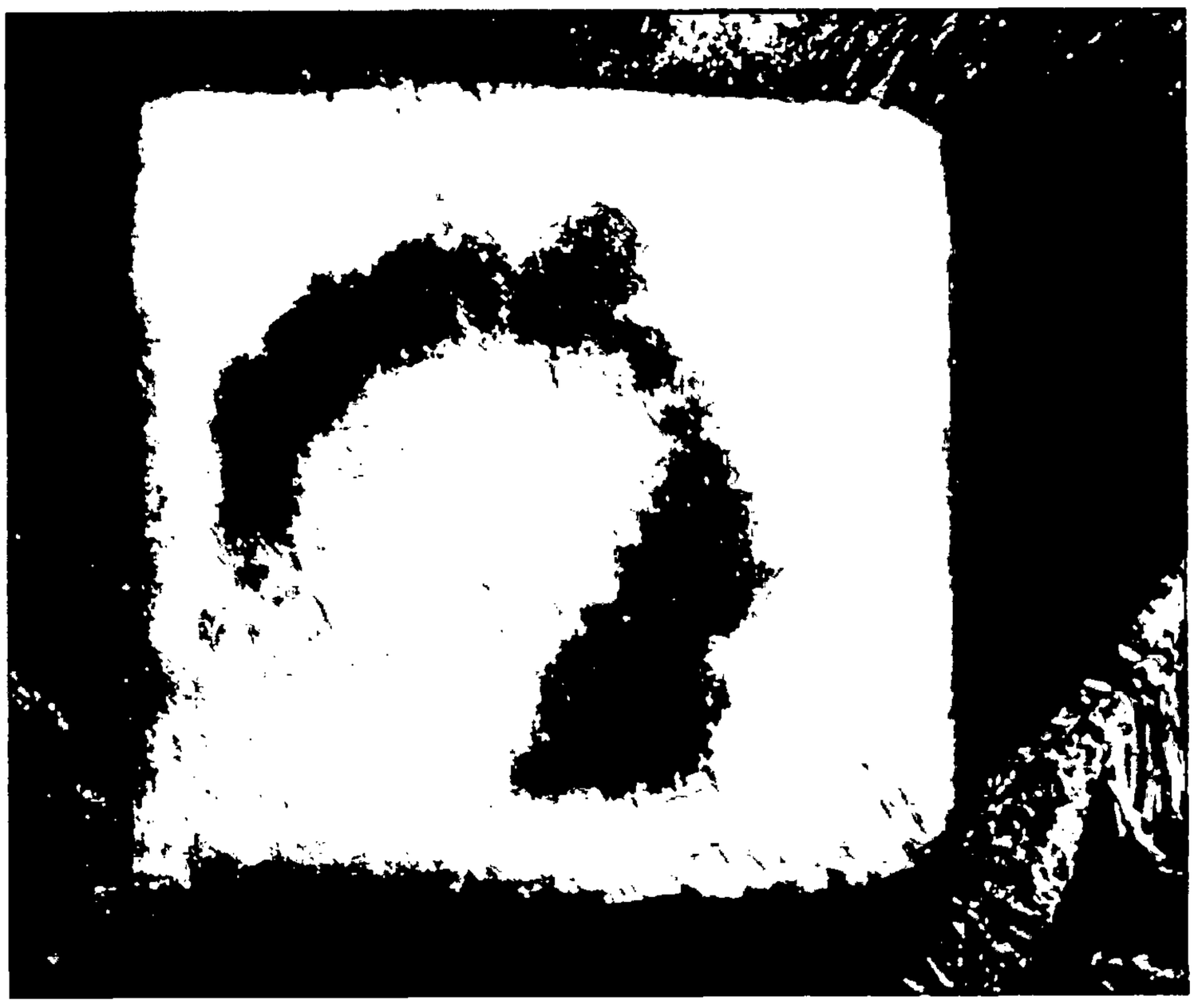

Figure 3

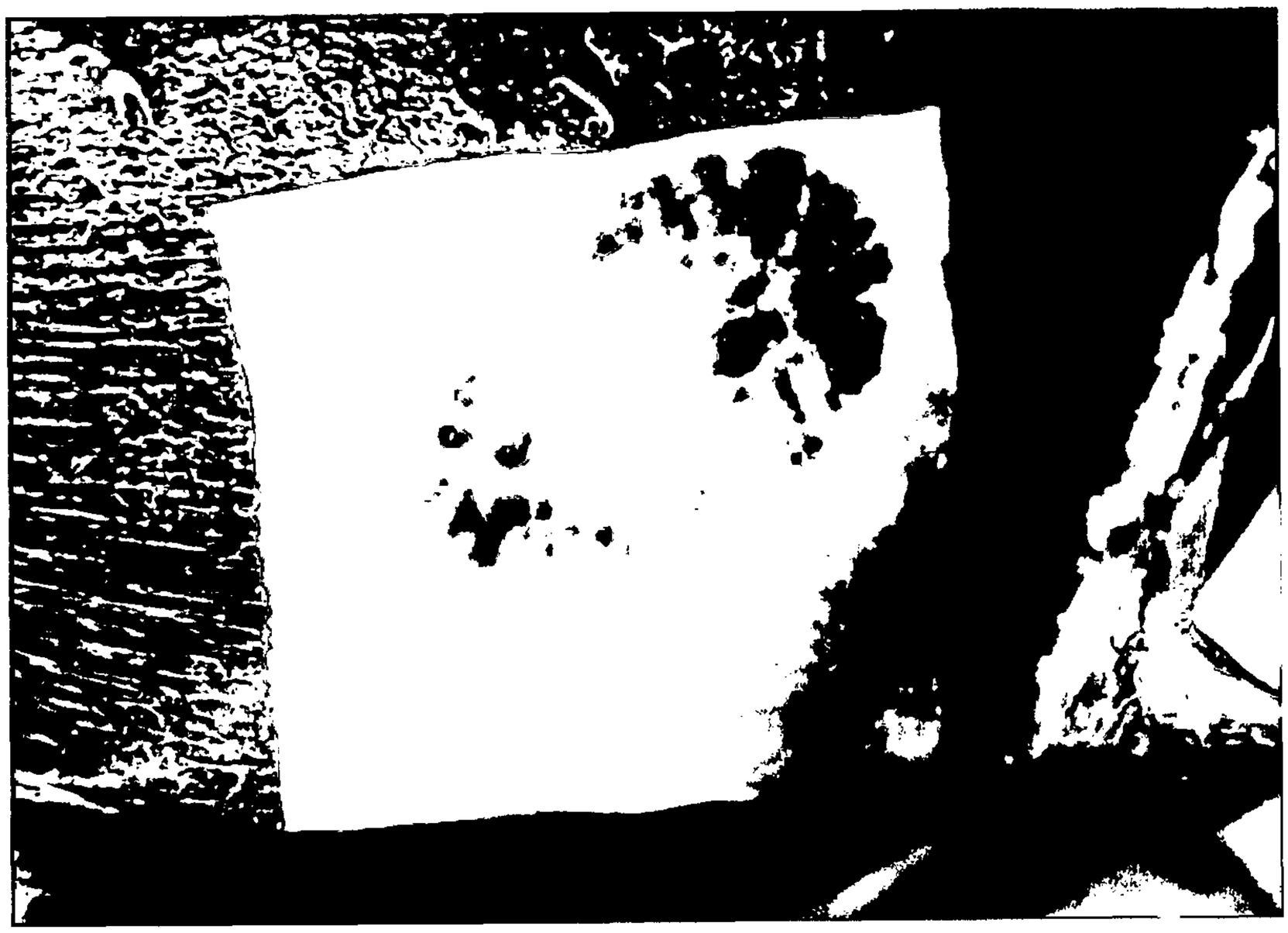

Figure 4