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(54) Benævnelse: **Vævsklæber, indeholdende fibrinogen, til hindring af vævsadhæsion.**

(56) Fremdragne publikationer:
WO-A-99/29338
DE IACO PIERANDREA ET AL.: "Fibrin sealant in laparoscopic adhesion prevention in the rabbit uterine horn model", FERTILITY AND STERILITY, Bd. 62, Nr. 2, 1. August 1994 (1994-08-01) , Seiten 400-404, XP001025656,
LINDENBERG S. ET AL.: "Prevention of peritoneal adhesion formation by fibrin sealant", ANNALES CHIRURGIAE ET GYNAECOLOGIAE, Bd. 73, Nr. 1, 1. Januar 1984 (1984-01-01) , Seiten 11-13, XP001025655,
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Cloth adhesive with improved anti-adhesive properties

Description

The invention relates to the use of a tissue adhesive for reducing or preventing post-operative tissue adhesions, which tissue adhesive is distinguished by improved anti-adhesive properties with respect to known tissue adhesives.

It is known that haemostatic efficacy or sealing (e.g. against loss of serous fluid) has so far been the focus in the development of tissue adhesives. These indications continue to account for the very substantial number of uses of tissue adhesives.

However, the preclinical or clinical use of tissue adhesives for avoiding adhesions after surgical procedures – with varying success – has also been described in the past. For instance, H. Moro et al. reported an inhibition of pericardial adhesions in a dog model (H. Moro, J. Hayashi, H. Ohzeki, T. Nakayama, O. Namura, K. Hanzawa and N. Yagi. Jap J Thor Cardiovasc Surg 47: 79–84, 1999). H. Takeuchi et al. and P.A. De Iaco et al. too described the successful use of tissue adhesives for avoiding or reducing adhesions at the uterine horn in rabbit (H. Takeuchi, Y. Toyonari, N. Mitsuhashi and Y. Kuwabara. J Obstet Gynaecol 23: 479–484, 1997; P.A. De Iaco, A. Costa, G. Mazzoleni, G. Pasquinelli, L. Bassein and A. Marabini. Fertil Steril 62: 400–404, 1994). S. Lindenberg et al. likewise described the reduction in peritoneal adhesions through the use of tissue adhesives in a rat model (S. Lindenberg and J.G. Lauritsen. Annales Chirurgiae et Gynecologiae 73: 11–13, 1984). However, there have also been other authors who, when using fibrin glues, were unable to observe any reduction in adhesions with respect to an untreated control. These included J.F.H. Gauwerki, J. Mann and G. Bastert, Arch Gynäkol Obstet 247: 161 (1990) and V.A.C. Evrard, A. De Bellis, W. Boeckx and I.A. Brosens, Hum Reprodt 11: 1877–1880 (1996). The most likely reason for the partly contradicting reports is that the effect achievable by the existing products is not large or distinct enough to consistently yield unambiguous results.

Recently, the possibility of using fibrin layers for avoiding adhesions has also been mentioned in the patent literature. International patent application WO 96/22115 describes a film-type material which consists of cross-linked fibrin and which is used for preventing adhesions, but itself has no haemostatic properties. In another embodiment, there is in situ generation of this material, in which it is used as a

second tissue-adhesive layer without any haemostatic properties after a first haemostatically effective tissue adhesive. However, these methods are either impracticable, since the fixation of such a fibrin film is difficult, or laborious, since two tissue adhesives must be used in order to achieve both haemostatic efficacy and anti-adhesive properties.

In addition, international patent application WO 92/22312 also discloses a preparation composed of fibrin or fibrinogen and a biocompatible or biodegradable, viscous-solution-forming polymer having anti-adhesive properties.

It is therefore an object to develop a tissue adhesive which exhibits good haemostatic properties coupled with improved results in the reduction or prevention of tissue adhesions and which, furthermore, manages without the addition of viscous-solution-forming polymers having anti-adhesive properties.

Because of their huge medical significance, considerable efforts have been made in research in recent years in order to further develop and improve the known tissue adhesives. In this connection, particular attention has also been paid to improving the shelf life of tissue adhesives. For instance, German patent applications DE-A-198 53 033, DE-A-198 61 158 and DE-A 100 12 732 have described tissue adhesives and the components thereof which are distinguished by, inter alia, an especially long shelf life in the liquid and/or frozen state. Closer investigation of these new tissue adhesives has now shown that they have yet further advantageous properties which open up to them additional and valuable possible uses.

Specifically, it has been found that these new tissue adhesives exhibit considerably improved anti-adhesive properties without losses in their haemostatic properties having to be tolerated at the same time. The special, anti-adhesive properties of the new tissue adhesives become apparent both in comparison with untreated wounds and in comparison with wounds treated with conventional tissue adhesives. In this connection, what is particularly surprising is that, even in the comparison with conventional tissue adhesives, distinctly improved effects are achieved in the reduction or prevention of tissue adhesions when the aforementioned new tissue adhesives are used. Said effects were observed both in a typical animal model for investigating reduction in adhesion, such as a longitudinal incision wound on the uterine horn in rabbit, and in the haemostatic application of a partial liver resection in rabbit.

The invention therefore provides for the use of a tissue adhesive containing

- a stabilized fibrinogen preparation storable in the liquid and/or frozen state, to which a chaotropic substance has been added, and
- a thrombin preparation

for reducing or preventing post-operative tissue adhesions.

At the same time, a blood coagulation factor XIII-containing preparation can be additionally added to the tissue adhesive if this is not present in a sufficient quantity, and so said tissue adhesive is used as a 3-component adhesive. This is because a maximally complete fibrin cross-linking can intensify the anti-adhesive effect of a fibrin glue, by the fibrin matrix being less accessible to, for example, a fibrinolytic degradation. However, it is also possible to add blood coagulation factor XIII to the fibrinogen preparation from the start, and so a 2-component adhesive is used. In the case of a 3-component adhesive, the mixture ratio of the components fibrinogen, factor XIII and thrombin can be appropriately selected in order to achieve good mechanical properties of the adhesive. Mixture ratios of approx. 1:1:1 to approx. 10:1:1 or 10:1:2 or generally x:y:z where $x \geq z \geq y$ are suitable for example.

The tissue adhesive used according to the invention contains a chaotropic substance in the fibrinogen preparation. Arginine, guanidine, citrulline, urea or the derivatives thereof or mixtures thereof have been found to be suitable chaotropic substances for example. They are added to the fibrinogen preparation generally in quantities of from 0.1 to 1.0 mol/l, preferably in quantities of below 0.5 mol/l.

The properties of the aforementioned new tissue adhesives are further advantageously influenced by the addition of an antifibrinolytic. The antifibrinolytic used is, for example, aprotinin, ε-aminocaproic acid (EACA), p-aminomethylbenzoic acid (PAMBA) or one of their physiologically tolerable salts or derivatives.

In addition, the fibrinogen preparation can contain as stabilizers

- an inorganic salt or
- one or more physiologically tolerable salts of organic carboxylic acids, more particularly of citric acid or lactic acid, or
- one or more amino acids or

- a monosaccharide or disaccharide or
- a sugar alcohol

or any mixture thereof.

A positive effect on the anti-adhesive properties of the claimed, improved fibrin glues can be further achieved by suitable purification methods, for example by reducing the plasminogen content of the fibrinogen component. Such methods can, for example, be immunoaffinity chromatography via coupled antibodies or affinity chromatography via amino group-containing matrices. Therefore, this invention also encompasses, inter alia, fibrin glues containing fibrinogen components, the plasminogen contents of which have been significantly reduced. Such fibrinogen components preferably have a ratio of plasminogen to fibrinogen of $< 1.8 \times 10^{-4}$ (w/w), particularly preferably of $< 10^{-4}$ (w/w).

The factor XIII preparation added to the tissue adhesive to be used according to the invention must likewise be stabilized, if said preparation is not added to the already stabilized fibrinogen. In this case, it is advantageous to add to the factor XIII preparation a physiologically tolerable salt of an organic di-, tri- or tetracarboxylic acid, particularly of citric acid, and optionally further stabilizers and/or buffer substances for factor XIII. Possible further stabilizers in this case are

- a monosaccharide or disaccharide or a sugar alcohol and/or
- an amino acid from the group consisting of glycine, glycylglycine, alanine, cysteine, histidine, glutamine or a physiologically tolerable salt of glutamic acid or aspartic acid and/or
- a reducing or oxidation-preventing agent and/or
- a surface-active substance.

They are customarily added to the factor XIII preparation in a quantity of up to 5% by weight. Tissue adhesives of this type are described in German patent applications DE-A-198 53 033 and DE-A-198 61 158.

In one embodiment, the thrombin preparation present in the tissue adhesive used according to the invention has the special feature of having the possibility of containing a non-covalent-binding inhibitor as stabilizer. Suitable substances for this purpose are compounds such as benzamidine or p-aminobenzamidine and other low-affinity to medium-affinity protease inhibitors. As a result of the addition of said low-affinity or medium-affinity inhibitors, the activity of thrombin with respect to substances such as fibrinogen is not substantially impaired. In addition to

a soluble calcium salt for stabilization, it is also possible to add to the thrombin preparation sodium chloride, a sugar or a sugar alcohol and/or an amino acid or else the salt of a monocarboxylic acid or polycarboxylic acid and/or the salt of a monohydroxycarboxylic acid or polyhydroxycarboxylic acid or mixtures of said stabilizers.

The thrombin used for this purpose is prepared from the prothrombin obtained from plasma or from a plasma fraction. After its activation to form thrombin without addition of thromboplastin and any further processing steps, it can be purified by means of a hydrophobic interaction chromatography and/or a cation-exchange chromatography. This method is described in detail in German patent application DE-A-100 12 732. At the same time, it is particularly advantageous when the tissue adhesive or its constituents are also additionally subjected to one or more methods for inactivating viruses.

As starting material for the preparation of the individual components of the fibrin glues according to the invention, it is possible to use not only plasma fractions, but also recombinant proteins, prepared by isolation from cell cultures or cell culture supernatants.

The effects of these improved tissue adhesives on the prevention or reduction of post-operative tissue adhesions were investigated by preparing, as an example, an improved tissue adhesive of the following composition:

Fibrinogen component containing:

90 mg/ml fibrinogen concentrate,
100 mmol/l NaCl,
20 mmol/l Na_3 citrate x $2H_2O$,
237 mmol/l L-arginine x HCl and
80 mmol/l ε-aminocaproic acid or 1000 KIU/ml aprotinin

Factor XIII component containing:

120 U/ml factor XIII concentrate,
10 mmol/l Na_3 citrate x $2H_2O$,
50 mmol/l L-histidine

Thrombin component containing:

1500 IU/ml thrombin concentrate,

150 mmol/l NaCl,
40 mmol/l $CaCl_2$,
110 mmol/l mannitol,
5 mmol/l L-histidine.

The pH after mixing of the components to form the tissue adhesive was approx. 7.4.

The use of said tissue adhesive in operations is exemplarily described below:

Example 1: Prevention of adhesions at the uterine horn.

After opening of the abdominal cavity of 12 anaesthetized female rabbits, longitudinal incisions were made on the uterine horns. The incisions were sealed using surgical suture material. Six rabbits were assigned to each of the following two treatment groups: 1. no treatment, 2. treatment with improved tissue adhesive. The wounds in the 2nd group were in each case completely covered with tissue adhesive. After closure of the abdominal cavity, the animals were able to wake up. After seven days, the rabbits were euthanized and the adhesions of the uteri with the surrounding tissue were assessed. The evaluation excluded cases where both incisions grew together. The results of the investigation are shown in Table 1.

The untreated animals exhibited adhesions in 63.6% of all cases. A distinct reduction in the adhesions was observed in the group treated with improved tissue adhesive. In this case, the frequency of the adhesions was just 11.1%.

Table 1: Uterine adhesions with the surrounding tissue after the treatment with tissue adhesives

	1. No treatment	2. Improved tissue adhesive
Frequency of the adhesions	63.6%	11.1%

Example 2: Prevention of adhesions at the uterine horn

In this experiment, the improved tissue adhesive was compared with a commercial adhesive (Beriplast® P) and with no treatment on 36 rabbits in total. In line

with the method described in Example 1, three groups were formed which each contained 12 animals and in which one uterine horn of each animal was treated as follows: 1. no treatment, 2. Beriplast® P, 3. improved tissue adhesive. The frequency and the extent of the adhesions were assessed on day 7. The results are compiled in Table 2.

All animals in the group which did not receive any treatment with a tissue adhesive exhibited adhesions (100%). The rabbits treated with Beriplast® P had a distinctly lower frequency of adhesions (75%). The lowest adhesion frequency was observed in the group of animals which were treated with improved tissue adhesive. The extent of the adhesions (length in cm) yielded similar findings.

Table 2: Uterine adhesion with the surrounding tissue after the treatment with fibrin glues

	1. No treatment	**2. Beriplast® P**	**3. Improved tissue adhesive**
Adhesion frequency (%)	100%	75%	50%
Length of the adhesions (cm)	1.52	1.03	0.67

Example 3: Prevention of adhesions at the uterine horn.

In a further experiment, improved tissue adhesives were compared with a commercial adhesive (Beriplast® P) and with an untreated control. In line with the method described in Example 2, several groups were formed, each containing 12 animals, with only one uterine horn being used per animal. The animals were treated as follows:

1. No treatment
2. Beriplast® P
3. Improved tissue adhesive
4. Improved tissue adhesive (aprotinin instead of EACA)
5. Improved tissue adhesive with reduced plasminogen content

The frequency and the extent of the adhesions were assessed on day 7. Table 3 shows the results of the study.

Approx. two thirds of the animals which did not receive any treatment with a tissue adhesive exhibited adhesions (66.7%). A distinctly lower frequency of adhesions or the lowest adhesion frequency was observed in the group of animals which were treated with improved tissue adhesives. The extent of the adhesions (length in cm) yielded similar findings.

Table 3: Adhesions of the uterus with the surrounding tissue after the treatment with different fibrin glues (mean values for n= 12 animals)

	1. Untreated control	2. Beriplast® P	3. Improved tissue adhesive	4. Improved tissue adhesive (aprotinin instead of EACA)	5. Improved tissue adhesive with reduced plasminogen content
Adhesion frequency (%)	66.7%	41.7%	33.3%	16.7%	0%
Length of the adhesions (cm)	0.59	0.19	0.26	0.13	0

Example 4: Prevention of adhesions at the uterine horn.

In this experiment, in line with the method described in Example 1, several groups were formed which each contained 8 animals and in which both uterine horns were operated on. Use was made of improved tissue adhesives together with a control group with no treatment, on 16 uterine horns per group. The following treatment groups were compared:

1. No treatment
2. Improved tissue adhesive with reduced plasminogen content
3. Improved tissue adhesive with plasminogen content that was initially reduced and was topped up before use

Only those uterine horns which did not adhere to the incision of the other uterine horn were evaluated. The results of this series of tests (see Table 4) show that the depletion of plasminogen can further improve the anti-adhesive properties of a fibrin glue.

Table 4: Prevention of adhesions at the uterine horn by treatment with fibrin glues (mean values)

	1. No treatment	2. Improved tissue adhesive with reduced plasminogen content	3. Improved tissue adhesive after reduction of the plasminogen content and topping up with plasminogen
Adhesion frequency (%)	68.8%	15.4%	46.2%
Length of the adhesions (cm)	0.53	0.07	0.32

Example 5: Prevention of adhesions after liver resection

14 rabbits were anaesthetized and the liver was visualized after opening of the abdominal cavity. From one liver lobe, a piece of approx. 3.5 g was resected, resulting in a wound of approx. 4 cm^2. The wound was completely covered with a tissue adhesive for the purposes of haemostasis, with seven rabbits receiving either Beriplast® P or improved tissue adhesive. The number of animals with complete haemostasis was determined over five minutes. Thereafter, the abdominal cavity was sealed and anaesthesia was brought to an end. After seven days, the animals were euthanized and the adhesions of the liver with the adjacent tissue were assessed.

Table 5 shows that the number of adhesions in the group which was treated with improved tissue adhesive was distinctly lower than in the group which had been treated with Beriplast® P. All animals exhibited a complete haemostasis.

Table 5: Haemostasis and adhesions of the liver with the surrounding tissue after the treatment with tissue adhesives

	1. Beriplast® P	2. Improved tissue adhesive
Number of animals with adhesions	5/7 (71.4%)	2/7 (28.6%)
Number of animals with complete haemostasis	7/7 (100%)	7/7 (100%)

PATENTKRAV

1. Vævsklæber, indeholdende:

   - Et stabiliseret fibrinogen-præparat, som kan lagres i flydende og/eller frosset tilstand, og som har fået tilsat en chaotropisk substans, og
   - et thrombin-præparat til anvendelse ved reduktion eller hindring af post-operativ vævsadhæsion.

2. Vævsklæber ifølge krav 1,
   k e n d e t e g n e t v e d , at den desuden omfatter et præparat, som indeholder blodkoaguleringsfaktor XIII, der også kan blandes sammen med fibrinogen-præparatet.

3. Vævsklæber ifølge krav 1 og krav 2,
   k e n d e t e g n e t v e d , at den indeholder et anti-fibrinolytikum.

4. Vævsklæber ifølge krav 3,
   k e n d e t e g n e t v e d , at den indeholder ε-aminosyre som anti-fibrinolytikum.

5. Vævsklæber ifølge krav 3,
   k e n d e t e g n e t v e d , at den indeholder p-amino-methylbenzoesyre som anti-fibrinolytikum.

6. Vævsklæber ifølge krav 3,
   k e n d e t e g n e t v e d , at den indeholder aprotinin som anti-fibrinolytikum.

7. Vævsklæber ifølge krav 1 til 6,
   k e n d e t e g n e t v e d , at indholdet af plasminogen i fibrinogen-komponenten er blevet reduceret ved hjælp af en immunoaffinitets-kromatografi via koblede antilegemer eller en affinitets-kromatografi via aminogruppe-indeholdende bærere.

8. Vævsklæber ifølge krav 1 til 7,

k e n d e t e g n e t v e d , at fibrinogen-komponenten omfatter et reduceret indhold af plasminogen, hvorved forholdet mellem plasminogen og fibrinogen er mindre end $1,8*10^{-4}$ vægt/vægt.

9. Vævsklæber ifølge krav 2 til 8,
k e n d e t e g n e t v e d , at det faktor XIII-indeholdende præparat har fået tilført:

- Et fysiologisk acceptabelt salt af en organisk di-, tri- eller tetracarbonsyre, og
- i givet fald yderligere stabilisatorer og/eller buffersubstanser for faktor XIII.

10. Vævsklæber ifølge krav 9,
k e n d e t e g n e t v e d , at det faktor XIII-indeholdende præparat som yderligere stabilisatorer har fået tilført:

- Et monosaccharid eller disaccharid
og/eller
- en aminosyre fra gruppen, som består af glycin, glycylglycin, alanin, cystein, histidin, glutamin eller et fysiologisk acceptabelt salt af glutaminsyre
og/eller
- et reducerende eller oxyderings-hindrende middel
og/eller
- en overflade-aktiv substans.

11. Vævsklæber ifølge krav 1 til 10,
k e n d e t e g n e t v e d , at fibrinogen-præparatet har fået tilført en eller flere chaotropiske substanser fra gruppen, der består af arginin, guanidin, citrullin, urea eller disses derivater eller blandinger af dem.

12. Vævsklæber ifølge krav 1 til 11,
k e n d e t e g n e t v e d , at fibrinogen-præparatet ydermere som stabilisatorer har fået tilføjet:

- Et uorganisk salt eller

- et eller flere fysiologisk acceptable salte af organiske carboxylsysrer, navnlig citronsyre eller mælkesyre, eller
- en eller flere aminosyrer, eller
- et monosaccharid eller disaccharid, eller
- en sukkeralkohol

eller en hvilken som helst blanding deraf.

13. Vævsklæber ifølge krav 1 til 12,
k e n d e t e g n e t v e d , at der anvendes et thrombin-præparat, som er stabilt i flydende eller i frossen tilstand, og som ud over et opløseligt calciumsalt og natriumchlorid som stabilisatorer kan indeholde:

- En buffer-substans
- et sukker eller en sukkeralkohol og/eller en aminosyre
  og/eller
- et salt af en monocarbolsyre eller polycarbolsyre
  eller
- et salt af en monohydroxy-carbolsyre eller polyhydroxy-carbolsyre eller blandinger af de nævnte stabilisatorer.

14. Vævsklæber ifølge krav 1 til 13,
k e n d e t e g n e t v e d , at thrombin-præparatet som stabilisator indeholder en ikke-covalent-bindende inhibitor.

15. Vævsklæber ifølge krav 1 til 14,
k e n d e t e g n e t v e d , at den indeholder et thrombin-præparat, som er renset ved hjælp af en hydrofob interaktionschromatografi og/eller en kation-udvekslingschromotografi.

16. Vævsklæber ifølge krav 1 til 15,
k e n d e t e g n e t v e d , at den eller dens bestanddele er blevet underkastet en eller flere fremgangsmåder til inaktivering eller fjernelse af vira.