[0004] TISSEEL® is presently commercially available in two different presentations; TISSEEL® Kit (Freeze Dried) and TISSEEL® Pre-filled Syringe (Frozen). TISSEEL® (Frozen) comprises a double-chamber syringe, pre-filled with Sealer Protein Solution and Thrombin Solution, that is thawed before use and then applied using the application device(s) supplied with the product. It is available in 2, 4, and 10 ml pack sizes.

[0005] TISSEEL® Pre-filled Syringe (Frozen) is stored at or below -20°C and must be thawed and warmed to 33 – 37 °C prior to use. The thawing at room temperature (15 to 25°C) can take 60 to 160 minutes depending on the syringe and size with an additional 2 to 52 minutes to warm depending on the syringe, packaging, size, and heat source. Combining thawing and warming, the product can take from 5 to 105 minutes depending on the syringe, packaging, size, and heat source to prepare. If the syringe is thawed at 15 to 25°C it must be warmed and used within 48 hours and once warmed at 33 – 37°C, it must be used within 4 hours.

[0006] These thawing and warming times require that surgical teams carefully plan each step involved in preparing TISSEEL®: removal from -20°C, transport to the operating room, thawing time at 15 to 25°C, removal of packaging, and warming (and thawing) time to 33 – 37°C. Further the availability of the warmed product must match the step of the surgical procedure due to the 4-hour warmed shelf-life, and once warmed, unused, unopened fibrin sealant must be discarded. Accordingly, there is an unmet need for fibrin sealants with improved usability (in particular with regard to usage timing flexibility) and for reduced waste of fibrinogen (which is a scarce human-donated material in case of the presently commercially available fibrin sealants).

[0007] It is thus an object of the present invention to provide improved fibrin sealant components, i.e. fibrinogen and thrombin formulations, which are more stable at room temperature in liquid form and/or which can be used by the surgeon with a reduced need for preparation steps (in particular without thawing of frozen and/or dissolution of lyophilized components, and/or with reduced need for warming at 33-37°C before use).

SUMMARY

[0008] The present disclosure provides a fibrinogen solution (for preparing a fibrin sealant) comprising fibrinogen at a concentration of at least 40 mg/ml, factor XIII, pharmaceutically acceptable additives and water. Typically, the dynamic viscosity of this solution measured at 20°C increases at most by 35% after storing the solution at 20°C for 30 days;

preferably, the dynamic viscosity of the solution measured at 20°C increases at most by 30%, preferably at most by 25%, more preferably at most by 20%, even more preferably at most by 15%, especially at most by 10% after storing the solution at 20°C for 30 days. In addition, or alternatively thereto, it is preferred that (immediately after thawing, i.e. before storing the solution in a liquid state e.g. for up to 30 days) the dynamic viscosity of the solution measured at 20°C is less than 120 mPa·s, preferably less than 110 mPa·s, more preferably less than 105 mPa·s, especially less than 100 mPa·s.

[0008a] In one aspect, the present disclosure provides a fibrinogen solution comprising fibrinogen at a concentration of at least 40 mg/ml, factor XIII, pharmaceutically acceptable additives and water, wherein the pharmaceutically acceptable additives comprise guanidine and arginine, wherein the sum of the guanidine concentration and the arginine concentration in the solution is between 5 mM and 200 mM, and wherein the dynamic viscosity of the solution measured at 20°C increases at most by 35% after storing the solution at 20°C for 30 days.

[0009] The present disclosure further relates to a thrombin solution (for preparing a fibrin sealant), comprising thrombin, pharmaceutically acceptable additives and water. Typically, the thrombin activity of this solution decreases at most by 15%, preferably at most by 12.5%, more preferably at most by 10%, even more preferably at most by 7.5%, especially at most by 5% after storing the solution at 25°C for 14 days. Alternatively, or in addition thereto, it is preferred that: the thrombin activity decreases at most by 25%, preferably at most by 20%, more preferably at most by 15% after storing the solution at 25°C for 28 days, or that the thrombin activity decreases at most by 30%, preferably at most by 25%, more preferably at most by 20%, even more preferably at most by 15% after storing the solution at 25°C for 35 days or that the thrombin activity decreases at most by 35%, preferably at most by 30%, more preferably at most by 25%, even more preferably at most by 20% after storing the solution at 25°C for 42 days; or that the thrombin activity decreases at most by 10%, preferably at most by 7.5% after storing the solution at 15°C for 42 days; or that the thrombin activity decreases at most by 35%, preferably at most by 30%, more preferably at most by 25%, even more preferably at most by 20%, especially at most by 15% after storing the solution at 30°C for 14 days, or that the thrombin activity

decreases at most by 50%, preferably at most by 45%, more preferably at most by 40%, even more preferably at most by 35%, especially at most by 30% or even at most by 25% after storing the solution at 30°C for 28 days, or that the thrombin activity decreases at most by 55%, preferably at most by 50%, more preferably at most by 45%, even more preferably at most by 40%, especially at most by 35% or even at most by 30% after storing the solution at 30°C for 35 days or that the thrombin activity decreases at most by 60%, preferably at most by 55%, more preferably at most by 50%, even more preferably at most by 45%, especially at most by 40% or even at most by 35% after storing the solution at 30°C for 42 days; or that the thrombin activity decreases at most by 55%, preferably at most by 50%, more preferably at most by 45%, even more preferably at most by 40%, especially

at most by 35% or even at most by 30% after storing the solution at 37°C for 7 days, or that the thrombin activity decreases at most by 85%, preferably at most by 80%, more preferably at most by 75%, even more preferably at most by 70%, especially at most by 65% or even at most by 60% or at most by 55% after storing the solution at 37°C for 14 days.

[0009a] In another aspect, the present disclosure provides a thrombin solution, comprising thrombin, pharmaceutically acceptable additives and water, wherein the pharmaceutically acceptable additives comprise a cyclodextrin, wherein the cyclodextrin concentration in the solution is between 0.1 mM and 100 mM, and wherein the thrombin activity decreases at most by 70% after storing the solution at 37°C for 14 days.

[0010] In the present disclosure, a fibrin sealant kit, comprising at least a first container comprising a fibrinogen solution, and a second container comprising a thrombin solution, is provided. The fibrinogen solution is defined as set forth herein and/or the thrombin solution is defined as set forth herein.

[0010a] In another aspect, the present disclosure provides a fibrin sealant kit, comprising at least:

a first container comprising a fibrinogen solution, and

a second container comprising a thrombin solution;

wherein the fibrinogen solution comprises the solution as described herein, and/or wherein the thrombin solution comprises the solution as described herein.

[0011] The present disclosure relates to a method for preparing a fibrin sealant, comprising the steps of obtaining a fibrin sealant kit as defined herein, wherein the fibrinogen solution and the thrombin solution are in a frozen state, thawing the fibrinogen solution and the thrombin solution, storing the fibrinogen solution and the thrombin solution at a temperature of 10°C – 37°C, preferably of 15°C – 30°C, more preferably 18°C-25°C for at least three days, preferably for at least four days, more preferably for at least five days, even more preferably for at least seven days, yet even more preferably for at least ten days, especially for at least fifteen days or even for at least twenty days, (e.g. for up to 30 days or for up to 42 days or for up to 50 days) and, after the storing, combining (e.g. mixing) the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

[0011a] In another aspect, the present disclosure provides a method for preparing a fibrin sealant, comprising the steps of:

obtaining a fibrin sealant kit as described herein, wherein the fibrinogen solution and the thrombin solution are in a frozen state;

thawing the fibrinogen solution and the thrombin solution;

storing the fibrinogen solution and the thrombin solution at a temperature of 10°C – 37°C for at least five days; then

combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

[0012] The present disclosure also relates to a method for preparing a fibrin sealant, comprising the steps of obtaining a fibrin sealant kit as defined herein, wherein the fibrinogen solution and the thrombin solution are in a frozen state, thawing the fibrinogen solution in the first container and the thrombin solution in the second container, storing the fibrinogen solution in the first container and the thrombin solution in the second container at a temperature of 10°C – 37°C, preferably of 15°C – 30°C, more preferably 18°C-25°C for at least three days, preferably for at least four days, more preferably for at least five days, even more preferably for at least seven days, yet even more preferably for at least ten days, especially for at least fifteen days or even for at least twenty days, (e.g. for up to 30 days or for up to 42 days or for up to 50 days) and, after the storing, opening the sterile package containing the first container and/or the second container, after the opening, combining (e.g. mixing) the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

[0012a] In another aspect, the present disclosure provides a method for preparing a fibrin sealant, comprising the steps of:

obtaining a fibrin sealant kit as described herein, wherein the fibrinogen solution and the thrombin solution are in a frozen state;

thawing the fibrinogen solution in the first container and the thrombin solution in the second container;

storing the fibrinogen solution in the first container and the thrombin solution in the second container at a temperature of 10°C – 37°C for at least three days;

after the storing, opening the sterile package containing the first container and/or the second container; and

after the opening, combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

[0013] The present disclosure relates to a method for preparing a fibrin sealant, comprising the steps of obtaining a first container comprising a lyophilized formulation comprising fibrinogen, factor XIII and pharmaceutically acceptable additives, and a second container comprising a lyophilized formulation comprising thrombin and pharmaceutically acceptable additives, reconstituting, by adding water or an aqueous solution, the formulation of the first container to obtain the fibrinogen solution as set forth herein, and the formulation of the second container to obtain the thrombin solution as set forth herein, storing the fibrinogen solution and the thrombin solution at a temperature of 10°C – 37°C, preferably of 15°C – 30°C, more preferably 18°C-25°C for at least three days, preferably for at least four days, more preferably for at least five days, even more preferably for at least seven days, yet even more preferably for at least ten days, especially for at least fifteen days or even for at least twenty days, (e.g. for up to 30 days or for up to 42 days or for up to 50 days) and, after the storing, combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

[0013a] In another aspect, the present disclosure provides a method for preparing a fibrin sealant, comprising the steps of

obtaining a first container comprising a lyophilized formulation comprising fibrinogen, factor XIII and pharmaceutically acceptable additives, and a second container comprising a lyophilized formulation comprising thrombin and pharmaceutically acceptable additives;

reconstituting, by adding water or an aqueous solution, the formulation of the first container to obtain the fibrinogen solution as described herein, and the formulation of the second container to obtain the thrombin solution as described herein,

storing the fibrinogen solution and the thrombin solution at a temperature of 10°C – 37°C for at least three days; and

after the storing, combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

[0014] A method for treating a wound, especially a bleeding wound, or tissue comprising the step of applying a fibrin sealant obtained by a method disclosed herein to the wound or tissue, is provided.

[0014a] In another aspect, the present disclosure provides use of a fibrin sealant obtained by the method as described herein in the manufacture of a medicament for treating a wound or tissue.

[0014b] In another aspect, the present disclosure provides a fibrin sealant obtained by the method as described herein for use in treating a wound or tissue.

[0015] Further, the present disclosure provides a method for treating a wound, especially a bleeding wound, or tissue comprising the steps of combining (e.g. mixing) a fibrinogen solution and a thrombin solution to obtain a fibrin sealant, wherein the fibrinogen solution is defined as set forth herein and/or wherein the thrombin solution is defined as set forth herein, and applying the fibrin sealant to the wound or tissue.

[0015a] In another aspect, the present disclosure provides use of a fibrin sealant in the manufacture of a medicament for treating a wound or tissue, wherein

the fibrin sealant is to be obtained by combining a fibrinogen solution and a thrombin solution, wherein the fibrinogen solution is as described herein and/or wherein the thrombin solution is as described herein.

[0015b] In another aspect, the present disclosure provides a fibrin sealant for use in treating a wound or tissue, wherein

the fibrin sealant is to be obtained by combining a fibrinogen solution and a thrombin solution, wherein the fibrinogen solution is as described herein and/or wherein the thrombin solution is as described herein.

[0016] Finally, the present disclosure provides the fibrinogen solution disclosed herein and/or the thrombin solution disclosed herein for use in a method as disclosed herein.

[0016a] In another aspect, the present disclosure provides the fibrinogen solution as described herein, for use in treating a wound or tissue, wherein

the fibrinogen solution is to be combined with a thrombin solution to obtain a fibrin sealant, wherein the thrombin solution is as described herein.

[0016b] In another aspect, the present disclosure provides the thrombin solution as described herein, for use in treating a wound or tissue, wherein

the thrombin solution is to be combined with a fibrinogen solution to obtain a fibrin sealant, wherein the fibrinogen solution is as described herein.

[0016c] In another aspect, the present disclosure provides use of a combination of a fibrinogen solution and a thrombin solution in the manufacture of a medicament, wherein the medicament is a fibrin sealant, for treating a wound or tissue, wherein the fibrinogen solution is as described herein and/or wherein the thrombin solution is as described herein.

[0017] In the course of the present disclosure, it was found that storing fibrinogen solutions of the prior art (e.g. as marketed under TISSEEL®) at non-refrigerated temperatures (e.g. room temperature) for up to 30 days lead to a viscosity increase which made subsequent handling steps more difficult. Accordingly, a screen for pharmaceutically acceptable additives at various concentrations was performed to find additives which reduce this viscosity increase, while maintaining other beneficial properties (such as being able to form an opaque clot when combined with the thrombin solution). The present disclosure, inter alia, relates to fibrinogen solutions with

additives found in this screen. In particular, guanidine and arginine turned out to be very suitable additives, even at low concentrations.

[0018] Further, a screen for pharmaceutically acceptable additives at various concentrations was performed in order to find additives which increase stability of the thrombin solution (i.e. reduce thrombin activity loss) at non-refrigerated temperatures (e.g. room temperature), while maintaining other beneficial properties and having high compatibility with the fibrinogen solutions developed in the course of the present disclosure. The present disclosure, inter alia, relates to thrombin solutions with additives found in this screen. In particular, guanidine and cyclodextrins turned out to be very suitable additives, even at low concentrations.

DETAILED DESCRIPTION

[0019] Viscosity of fibrinogen solutions was discussed in the prior art, for instance in US 6447774 B1 which relates to stabilized protein preparations for a tissue adhesive. However, apparently no efforts were made to optimize the fibrinogen solution for longer-term storage at elevated temperatures (i.e. non-refrigerated). Accordingly, the authors of US 6447774 B1 could not observe that a dynamic viscosity increase was a problem also for prolonged storage at higher temperatures (under which dynamic viscosity of fibrinogen solutions is considerably lower, in general). Furthermore, it was suggested in the document to provide factor XIII as a separate component to increase stability of the fibrinogen solution. By contrast, it turned out in the course of the present invention that factor XIII can remain as a component in the fibrinogen solution and a room-temperature stable fibrinogen solution with excellent handling properties could nevertheless be achieved.

[0020] JP 2000229882 A relates to stabilization of aqueous solutions of thrombin. Cyclodextrin is disclosed as a stabilizer. However, stability tests are performed only at 5 °C. The document is silent with respect to stability of thrombin solutions at elevated temperatures such as room temperature.

[0021] Previous attempts to stabilize thrombin have also focused on reversibly inhibiting thrombin through pH [Fenton et al., J. Biol. Chem. 252 (11) 3587-3598. 1977], arginine or ammonium compounds [Laurent et al., Bioorganic & Medicinal Chemistry. 3 (8) 1145 – 1156. 1995], or pharmacologic agents [U.S. 10,131,896; U.S. 7,351,561; Markwardt et al., European J. Biochem. 6 (1968) 502-506. 1968]. EP 1649867A1 discloses a composition comprising purified

CLAIMS

1. A fibrinogen solution comprising fibrinogen at a concentration of at least 40 mg/ml, factor XIII, pharmaceutically acceptable additives and water, wherein the pharmaceutically acceptable additives comprise guanidine and arginine, wherein the sum of the guanidine concentration and the arginine concentration in the solution is between 5 mM and 200 mM, and wherein the dynamic viscosity of the solution measured at 20°C increases at most by 35% after storing the solution at 20°C for 30 days.

2. The fibrinogen solution of claim 1, wherein the guanidine concentration in the solution is between 3 mM and 200 mM.

3. The fibrinogen solution of claim 1 or 2, wherein the arginine concentration in the solution is between 0.5 mM and 90 mM.

4. The fibrinogen solution of any one of claims 1-3, wherein the pharmaceutically acceptable additives comprise sodium chloride; wherein the sodium chloride concentration in the solution is between 10 mM and 200 mM.

5. The fibrinogen solution of any one of claims 1-4, wherein the pharmaceutically acceptable additives comprise histidine; wherein the histidine concentration in the solution is between 25 mM and 250 mM.

6. The fibrinogen solution of any one of claims 1-5, wherein the dynamic viscosity of the solution measured at 20°C is less than 120 mPa·s.

7. The fibrinogen solution of any one of claims 1-6, wherein the osmolality of the solution is between 300 mOsmol/kg and 700 mOsmol/kg.

8. The fibrinogen solution of any one of claims 1-7, wherein the solution comprises fibrinogen at a concentration of between 50 mg/ml and 150 mg/ml.

9. The fibrinogen solution of any one of claims 1-8, wherein the dynamic viscosity of the solution measured at 20°C increases at most by 30% after storing the solution at 20°C for 30 days.

10. The fibrinogen solution of any one of claims 1-9, wherein the solution comprises factor XIII at a concentration of at least 0.1 IU/ml.

11. The fibrinogen solution of any one of claims 1-10, wherein the solution further comprises aprotinin, at a concentration of between 250 KIU/ml and 4000 KIU/ml.

12. The fibrinogen solution of any one of claims 1-11, wherein the pharmaceutically

acceptable additives comprise at least one of albumin, citrate, surfactant and nicotinamide.

13. A thrombin solution, comprising thrombin, pharmaceutically acceptable additives and water, wherein the pharmaceutically acceptable additives comprise a cyclodextrin, wherein the cyclodextrin concentration in the solution is between 0.1 mM and 100 mM, and wherein the thrombin activity decreases at most by 70% after storing the solution at 37°C for 14 days.

14. The thrombin solution of claim 13, wherein the pharmaceutically acceptable additives further comprise guanidine; wherein the guanidine concentration in the solution is between 1 mM and 250 mM.

15. The thrombin solution of any one of claims 13 to 14, wherein the pharmaceutically acceptable additives comprise guanidine and a cyclodextrin, wherein the molar ratio between guanidine and cyclodextrin in the solution is between 20:1 and 1:4.

16. The thrombin solution of any one of claims 13 to 15, wherein the pharmaceutically acceptable additives further comprise histidine; wherein the histidine concentration in the solution is between 1 mM and 100 mM.

17. The thrombin solution of any one of claims 13 to 16, wherein the pharmaceutically acceptable additives further comprise methionine; wherein the methionine concentration in the solution is between 1 mM and 50 mM.

18. The thrombin solution of any one of claims 13 to 17, wherein the pharmaceutically acceptable additives further comprise glycine; wherein the glycine concentration in the solution is between 25 mM and 400 mM.

19. The thrombin solution of any one of claims 13 to 18, wherein the pharmaceutically acceptable additives further comprise alanine; wherein the alanine concentration in the solution is between 10 mM and 200 mM.

20. The thrombin solution of any one of claims 13 to 19, wherein the pharmaceutically acceptable additives further comprise trehalose; wherein the trehalose concentration in the solution is between 10 mM and 100 mM.

21. The thrombin solution of any one of claims 13 to 20, wherein the pH of the solution is 6.0-8.0.

22. The thrombin solution of any one of claims 13 to 21, wherein the osmolality of the solution is between 300 mOsmol/kg and 850 mOsmol/kg.

23. The thrombin solution of any one of claims 13 to 22, wherein the thrombin activity

is between 100 IU/ml and 2000 IU/ml.

24. The thrombin solution of any one of claims 13 to 22, wherein the pharmaceutically acceptable additives comprise at least one of, preferably at least two of, especially all of: a calcium salt such as calcium chloride, albumin and sodium chloride.

25. A fibrin sealant kit, comprising at least:

a first container comprising a fibrinogen solution, and

a second container comprising a thrombin solution;

wherein the fibrinogen solution comprises the solution of any one of claims 1 to 12, and/or wherein the thrombin solution comprises the solution of any one of claims 13 to 24.

26. The fibrin sealant kit of claim 25, further comprising a fibrin sealant delivery device.

27. The fibrin sealant kit of claim 25 or 26, wherein the first container and/or the second container is a pre-filled syringe.

28. The fibrin sealant kit of any one of claims 25 to 27, wherein the first container and/or the second container is contained within a sterile package.

29. A method for preparing a fibrin sealant, comprising the steps of:

obtaining a fibrin sealant kit as defined in any one of claims 25 to 28, wherein the fibrinogen solution and the thrombin solution are in a frozen state;

thawing the fibrinogen solution and the thrombin solution;

storing the fibrinogen solution and the thrombin solution at a temperature of 10°C – 37°C for at least five days; then

combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

30. A method for preparing a fibrin sealant, comprising the steps of:

obtaining a fibrin sealant kit as defined in claim 28, wherein the fibrinogen solution and the thrombin solution are in a frozen state;

thawing the fibrinogen solution in the first container and the thrombin solution in the second container;

storing the fibrinogen solution in the first container and the thrombin solution in the second container at a temperature of 10°C – 37°C for at least three days;

after the storing, opening the sterile package containing the first container and/or the second container; and

after the opening, combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

31. A method for preparing a fibrin sealant, comprising the steps of

obtaining a first container comprising a lyophilized formulation comprising fibrinogen, factor XIII and pharmaceutically acceptable additives, and a second container comprising a lyophilized formulation comprising thrombin and pharmaceutically acceptable additives;

reconstituting, by adding water or an aqueous solution, the formulation of the first container to obtain the fibrinogen solution of any one of claims 1 to 12, and the formulation of the second container to obtain the thrombin solution of any one of claims 13 to 24,

storing the fibrinogen solution and the thrombin solution at a temperature of 10°C – 37°C for at least three days; and

after the storing, combining the fibrinogen solution and the thrombin solution to obtain the fibrin sealant.

32. Use of a fibrin sealant obtained by the method of claim 29 or 30 or 31 in the manufacture of a medicament for treating a wound or tissue.

33. A fibrin sealant obtained by the method of claim 29 or 30 or 31 for use in treating a wound or tissue.

34. Use of a fibrin sealant in the manufacture of a medicament for treating a wound or tissue, wherein

the fibrin sealant is to be obtained by combining a fibrinogen solution and a thrombin solution, wherein the fibrinogen solution is defined as set forth in any one of claims 1 to 12 and/or wherein the thrombin solution is defined as set forth in any one of claims 13 to 24.

35. A fibrin sealant for use in treating a wound or tissue, wherein

the fibrin sealant is to be obtained by combining a fibrinogen solution and a thrombin solution, wherein the fibrinogen solution is defined as set forth in any one of claims 1 to 12 and/or wherein the thrombin solution is defined as set forth in any one of claims 13 to 24.

36. The fibrinogen solution of any one of claims 1 to 12, for use in treating a wound or tissue, wherein

the fibrinogen solution is to be combined with a thrombin solution to obtain a fibrin sealant, wherein the thrombin solution is defined as set forth in any one of claims 13 to 24.

37. The thrombin solution of any one of claims 13 to 24, for use in treating a wound or

tissue, wherein

the thrombin solution is to be combined with a fibrinogen solution to obtain a fibrin sealant, wherein the fibrinogen solution is defined as set forth in any one of claims 1 to 12.

38. Use of a combination of a fibrinogen solution and a thrombin solution in the manufacture of a medicament, wherein the medicament is a fibrin sealant, for treating a wound or tissue, wherein the fibrinogen solution is defined as set forth in any one of claims 1 to 12 and/or wherein the thrombin solution is defined as set forth in any one of claims 13 to 24.