

CLAIMS:

1. A method of preparing an isolated serum fraction of platelet rich fibrin (PRF), comprising the steps of
 - a. providing platelet rich plasma (PRP) without the addition of an anticoagulant;
 - b. clotting the PRP to obtain a coagel of PRF spontaneously by centrifugation carried out at 1000 to 5000 g;
 - c. pressing or squeezing the coagel to separate a serum fraction which comprises an activated platelet releasate from the coagel, thereby obtaining an isolated serum fraction containing the fluid fraction of PRF; and
 - d. providing the serum fraction in a pharmaceutical preparation which is contained in an application device for administration of the serum fraction to an individual.
2. The method of claim 1, wherein the centrifugation is carried out for 5 to 10 minutes.
3. The method according to any one of claims 1 or 2, wherein the PRP (step a) is prepared by separating the red blood cell fraction from a venous blood sample.
4. The method according to any one of claims 1 to 3, wherein the PRP is obtained from venous blood collected from a single donor.
5. The method according to any one of claims 1 to 4, wherein the serum fraction is prepared in a period up to 10 hours without adding preservatives.
6. The method according to any one of claims 1 to 5, wherein the isolated serum fraction contains at least one of angiogenesis or growth factor proteins selected from the group consisting of Activin-A, ADAMTS-1, Angiogenin, CXCL16, DPPIV, Endoglin, Endostatin/Collagen XVII, FGF-4, GM-CSF, HB-EGF, HGF, IGFBP-1, IGFBP-2, IGFBP-3, IL-1b, IL-8, LAP (TGFb-1), Leptin, MCP-1, MMP-8, MMP-9, NRG1-b1, Pentraxin-3, PD-ECGF, PDGF-AB/PDGF-BB, PIGF, Prolactin, TIMP-4, Thrombospondin-1, and uPA.
7. The method according to any one of claims 1 to 6, wherein in the isolated serum fraction wherein Platelet factor 4, Serpin EI, or TIMP-1 is at least 1.2-fold enriched as compared to PRP or whole blood.

8. The method according to any one of claims 1 to 7, wherein the isolated serum fraction is depleted in at least one of angiogenesis or growth factor related proteins selected from the group consisting of SDF-1, Angiopoietin-I, EGF, PDGF, VEGF, as compared to PRP or whole blood, wherein SDF-1 concentration is less than 50% (w/w) of the concentration as compared to PRP or whole blood, and which comprises a reduced content of red blood cells, platelets or fibrinogen as compared to whole blood.

9. The method according to any one of claims 1 to 8, wherein the application device is used as a preparation device both for collecting blood and for preparing PRP.

10. The method according to any one of claims 1 to 9, wherein the application device is a syringe and/or a bone graft material.

11. The method according to any one of claims 1 to 10, wherein the serum fraction includes supernatant of the coagel.

12. The method according to any one of claims 1 to 11, wherein the serum fraction prepared ready-to-use for the purpose of treating a patient without using a preservative.

13. The method according to any one of claims 1 to 12, further comprising the following steps carried out before step a):

a1) obtaining venous blood sample from an individual without anticoagulants,
a2) removing red blood cells from the blood sample,
wherein the venous blood sample is obtained from the individual to whom the serum fraction is to be administered.

14. The method according to any one of claims 1 to 13, wherein the PRP clotting (step b) is accelerated upon contact with negatively charged surfaces and without adding exogenous coagulation activators.

15. An isolated serum fraction obtained by the method of any one of claims 1 to 14.

16. A serum fraction as claimed in claim 15 when used in treating a patient suffering from osteonecrosis, osteoarthritis, osteoarthrosis, bone necrosis, tendinosis, critical limb ischemia, Buerger's disease, impingement syndrome, or a patient undergoing treatment

for dermal filling, rejuvenation of the nasolabial crest or any other facial wrinkles, bone grafting or implantation.

17. A serum fraction as claimed in claim 15 when used as a cell culture additive or to prepare bones or implants, specifically by surface treatment or coating.

18. A pharmaceutical preparation comprising a blood-derived serum product which is an isolated serum fraction of platelet rich fibrin (PRF) said a blood-derived serum product being contained in an application device for administration of the serum fraction to an individual and being obtained by method comprising the steps of

a. separating and removing the red blood cell fraction from a venous blood sample without the addition of an anticoagulant to provide a plasma having a platelet concentration above baseline;

b. clotting said plasma to obtain a coagel of PRF spontaneously by centrifugation carried out at 1000 to 5000 g;

c. pressing or squeezing the coagel to separate a serum fraction which comprises an activated platelet releasate from the coagel, thereby obtaining an isolated serum fraction containing the fluid fraction of PRF; and

d. providing the serum fraction in a pharmaceutical preparation which is contained in an application device for administration of the serum fraction to an individual,

said serum fraction comprising a platelet releasate from activated platelets.

19. The pharmaceutical preparation of claim 18, wherein in said method the centrifugation is carried out for 5 to 10 minutes.

20. The pharmaceutical preparation according to any one of claims 18 or 19, wherein in said method the centrifugation is carried out at 1600 to 2000 g.

21. The pharmaceutical preparation according to any one of claims 21 to 23, wherein in said method the centrifugation is carried out within 20 minutes from collecting venous blood.

22. The pharmaceutical preparation according to any one of claims 18 to 21, wherein the PRF is obtained from venous blood collected from a single donor.

23. The pharmaceutical preparation according to any one of claims 18 to 22, wherein the serum fraction is prepared in a period up to 10 hours without adding preservatives.

24. The pharmaceutical preparation according to any one of claims 18 to 23, wherein said serum fraction of PRF has a non-inflammatory, non-differentiating blood factor profile which induces cell proliferation.

25. The pharmaceutical preparation according to any one of claims 18 to 24, wherein said serum fraction comprises the fluid fraction of PRF.

26. The pharmaceutical preparation according to claim 25, wherein said serum fraction has a non-differentiating but cell proliferating profile on osteoblasts.

27. The pharmaceutical preparation according to any one of claims 25 or 26, which contains at least one of angiogenesis or growth factor proteins selected from the group consisting of Activin-A, ADAMTS-1, Angiogenin, CXCL16, DPPIV, Endoglin, Endostatin/Collagen XVII, FGF-4, GM-CSF, HB-EGF, HGF, IGFBP-1, IGFBP-2, IGFBP-3, IL-1b, IL-8, LAP (TGFb-1), Leptin, MCP-1, MMP-8, MMP-9, NRG1-b1, Pentraxin-3, PD-ECGF, PDGF-AB/PDGF-BB, PIGF, Prolactin, TIMP-4, Thrombospondin-1, and uPA.

28. The pharmaceutical preparation according to any one of claims 25 to 27, wherein Platelet factor 4, Serpin EI, or TIMP-1 is at least 1.2-fold enriched as compared to said plasma having a platelet concentration above baseline or whole blood.

29. The pharmaceutical preparation according to any one of claims 25 to 28, which is depleted in at least one of angiogenesis or growth factor related proteins selected from the group consisting of SDF-1, Angiopoietin-I, EGF, PDGF, VEGF, as compared to said plasma having a platelet concentration above baseline as provided in step a) or whole blood, wherein SDF-1 concentration is less than 50% (w/w) of the concentration as compared to said plasma having a platelet concentration above baseline or whole blood, and which comprises a reduced content of red blood cells, platelets or fibrinogen as compared to whole blood or a reduced content of fibrin as compared to said plasma having a platelet concentration above baseline.

30. The pharmaceutical preparation according to any one of claims 18 to 29, said pharmaceutical preparation comprising a pharmaceutically acceptable carrier and an additional active substance and/or device to promote wound healing, cell proliferation or regeneration, wherein the additional active substance is a hydrogel, a tissue sealant or an active component thereof, wherein the device is a bone graft material and which

preparation is contained in a syringe.

31. The pharmaceutical preparation of claim 30, wherein the application device is also used as a preparation device both for collecting blood and for preparing the serum fraction.

32. The pharmaceutical preparation according to any one of claims 18 to 31, wherein the application device is a syringe and/or a bone graft material.

33. The pharmaceutical preparation according to any one of claims 18 to 32, wherein the serum fraction includes supernatant of the coagel.

34. The pharmaceutical preparation according to any one of claims 18 to 33, wherein the serum fraction prepared ready-to-use for the purpose of treating a patient without using a preservative.

35. The pharmaceutical preparation according to any one of claims 18 to 34, wherein the freshly prepared serum fraction is storage stable frozen

- at 2°C - 12°C for up to 24 months, or
- at -80°C to -25°C for up to 5 years.

36. The pharmaceutical preparation of any one of claims 21 to 35 for in vitro use as a cell culture additive or to prepare bones or implants, specifically by surface treatment or coating.

37. An application kit comprising the components

- a. the pharmaceutical preparation according to any one of claims 21 to 35;
- b. an application device,

wherein the application device is a bone graft material, a gelling agent, a tissue sealant or an active component thereof; and

optionally the application kit also comprises a device for mixing the pharmaceutical preparation and the application device to obtain a mixture ready for application.