inconvenient, clinicians must centrifuge blood to isolate platelets from blood, and the variability of this process, which can last between 25-30 minutes, again introduces errors; and the current processes are not cost-effective with an estimated cost of $130 per treatment (Plateltex). The invention is a non-obvious solution to these long standing and persistent problems.

DESCRIPTION OF THE FIGURES

Fig. 1 shows a flowchart of the protocol used to produce a platelet lystate of the invention and a pharmaceutical composition of the invention.

Figs. 2 and 3 show that the invention provides a cost-effective, differentiated product that is convenient, consistent and cGMP compliant

Figs. 4 to 7 show that the solves the long standing and presistent need to provide a consistent wound care product that is quality controlled, convenient to use and cost effective.

SUMMARY

The present disclosure provides a pharmaceutical composition comprising a platelet lysate. These may be therapeutically applied to wounds, such as ulcerated wounds, anal fissures, vaginal atrophy or wrinkles.

The inventors have also devised a more efficient way of lysing platelets such they release their growth factors into solution as a platelet lysate. The novel platelet lysate may itself be used as a therapeutic or may be formulated into a pharmaceutical composition of the present disclosure.

In one aspect of the present disclosure, there is provided a use of a human platelet lysate in the manufacture of a pharmaceutical composition for the treatment of a wound, an anal fissure, vaginal atrophy or a wrinkle, wherein the human platelet lysate is produced by subjecting a population of human platelets to four freeze-thaw cycles, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to about -190 °C.

In another aspect of the present disclosure, there is provided a human platelet lysate for use in treating a wound, an anal fissure, vaginal atrophy or a wrinkle in a patient in need thereof, wherein the human platelet lysate is produced by subjecting a population of human

platelets to four freeze-thaw cycles, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to about -190 °C.

In a further aspect of the present disclosure, there is provided a method of producing a pharmaceutical composition comprising platelet lysate comprising (a) subjecting a population of platelets to four freeze-thaw cycles, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to about -190 °C, and (b) mixing the platelet lysate with at least one pharmaceutically acceptable polymer and at least one pharmaceutically acceptable positively charged chemical species selected from the group consisting of lysine, arginine, histidine, aspartic acid, glutamic acid, alanine, methionine, proline, serine, asparagine, cysteine, polyamino acids, protamine, aminoguanidine, zinc ions and magnesium ions such that the resulting composition is an aqueous gel having a viscosity in the range of 1000 to 500,000 mPa•s (cps) at room temperature.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising platelet lysate produced using a method disclosed herein.

In a further aspect of the present disclosure, there is provided a pharmaceutical composition disclosed herein for use in treating a wound, an anal fissure, vaginal atrophy or a wrinkle in a patient in need thereof.

In another aspect of the present disclosure, there is provided a use of a pharmaceutical composition disclosed herein in the manufacture of a pharmaceutical composition for the treatment of a wound, an anal fissure, vaginal atrophy or a wrinkle.

The composition or lysate of the present disclosure may be lyophilised to produce a stabilized, freeze-dried powder that contains these growth factors and which can be used for therapeutic application in wound healing, but also other therapeutic applications in which there is an increased need for delivery of natural growth factors.

The present disclosure provides a pharmaceutical composition comprising (a) a therapeutic platelet lysate, (b) at least one pharmaceutically acceptable polymer and (c) at least one pharmaceutically acceptable positively charged chemical species selected from the group consisting of lysine, arginine, histidine, aspartic acid, glutamic acid, alanine, methionine, proline, serine, asparagine, cysteine, polyamino acids, protamine, aminoguanidine, zinc ions

and magnesium ions, wherein the composition is an aqueous gel having a viscosity in the range of 1000 to 500,000 mPa•s (cps) at room temperature.

The present disclosure also provides:

- a method of producing a platelet lysate comprising subjecting a population of platelets to at least one freeze-thaw cycle, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to - 78 °C;
- a method of producing platelet lysate comprising subjecting a population of platelets to mechanical homogenisation ('waring blender'), liquid homogenization, sonication, freeze-thaw using alcohol and dry ice or other means, mechanical grinding in liquid nitrogen at >75%, but typically >90%, lysis to release growth factors from platelets and alpha granules into solution whereas rotating blades grind and disperse cells and tissues; cell or tissue suspensions are sheared by forcing them through a narrow space; high frequency sound waves shear cells; repeated cycles of freezing and thawing disrupt cells through ice crystal formation; grinding tissue, frozen in liquid nitrogen wherein lysis releases growth factors from platelets and alpha granules into solution;
- a platelet lysate produced using a method of the present disclosure;
- a pharmaceutical composition of the present disclosure or a platelet lysate of the present disclosure for use in treating a wound, an anal fissure, vaginal atrophy or a wrinkle in a patient in need thereof;
- a method of treating a wound, an anal fissure, vaginal atrophy or a wrinkle in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of a pharmaceutical composition of the present disclosure or a platelet lysate of the present disclosure;
- a method of producing a pharmaceutical composition of the present disclosure comprising mixing a platelet lysate with at least one pharmaceutically acceptable polymer and at least one pharmaceutically acceptable positively charged chemical species such that the resulting composition is an aqueous gel having a viscosity in the range of 1000 to 500,000 mPa•s (cps) at room temperature; and
- a method of producing a pharmaceutical composition of the present disclosure comprising (a) producing a platelet lysate using a method according to any one of claims 14 to 20 and (b) mixing the platelet lysate with at least one pharmaceutically acceptable polymer

CLAIMS

1. Use of a human platelet lysate in the manufacture of a pharmaceutical composition for the treatment of a wound, an anal fissure, vaginal atrophy or a wrinkle, wherein the human platelet lysate is produced by subjecting a population of human platelets to four freeze-thaw cycles, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to about -190 °C.

2. A human platelet lysate for use in treating a wound, an anal fissure, vaginal atrophy or a wrinkle in a patient in need thereof, wherein the human platelet lysate is produced by subjecting a population of human platelets to four freeze-thaw cycles, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to about -190 °C.

3. A method of producing a pharmaceutical composition comprising platelet lysate comprising (a) subjecting a population of platelets to four freeze-thaw cycles, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to about -190 °C, and (b) mixing the platelet lysate with at least one pharmaceutically acceptable polymer and at least one pharmaceutically acceptable positively charged chemical species selected from the group consisting of lysine, arginine, histidine, aspartic acid, glutamic acid, alanine, methionine, proline, serine, asparagine, cysteine, polyamino acids, protamine, aminoguanidine, zinc ions and magnesium ions such that the resulting composition is an aqueous gel having a viscosity in the range of 1000 to 500,000 mPa•s (cps) at room temperature.

4. A method according to claim 3, wherein liquid nitrogen is used as a cryogenic means whereby immersion in the freeze portion of each cycle results in 95% or more lysis of platelets resulting in greater growth factor release and improved function in cell growth, repair and regeneration measurable by but not limited to fibroblast and PGDF assay.

5. A method according to any one of claims 3 or 4, wherein the platelet lysate concentration is within the range of 0.1 µg to 1,000 µg per gram of composition.

6. A method according to any one of claims 3 to 5, wherein the polymer is (i) a cellulose polymer or (ii) carboxymethylcellulose, hydroxypropylmethylcellulose or methylcellulose.

7. A method according to claim 6, wherein the cellulose polymer concentration is 1.5% (w/w) to 4.0% (w/w) and the polymer has a molecular weight of 450,000 to 4,000,000.

8. A method according to any one of claims 3 to 7, wherein the polymer is a pluronic acid or Pluronic F-127.

9. A method according to any one of claim 8, wherein the polymer concentration is 15% (w/w) to 30% (w/w).

10. A method according to any one of the claims 3 to 9, wherein the concentration of the charged species is within the range of 0.1% (w/w) to 3.0% (w/w).
11. A method according to any one of claims 3 to 10, wherein the viscosity is in the range of 50,000 to 150,000 mPa•s(cps) at 25°C.

12. A method according to any one of claims 3 to 11, wherein step (b) further comprises mixing the platelet lysate with a preservative selected from the group consisting of methylparaben, propylparaben and m-cresol.

13. A method according to any one of claims 3 to 12, wherein the platelet lysate is from humans.

14. A pharmaceutical composition comprising platelet lysate produced using a method according to any one of claims 3 to 13.

15. A pharmaceutical composition according to claim 14 for use in treating a wound, an anal fissure, vaginal atrophy or a wrinkle in a patient in need thereof.

16. Use of a pharmaceutical composition according to claim 14 in the manufacture of a pharmaceutical composition for the treatment of a wound, an anal fissure, vaginal atrophy or a wrinkle.