

CLAIMS

1. A method of producing an acellular bioabsorbable tissue regeneration matrix, the method comprising:

(a) providing an isolated blood sample;

5 (b) obtaining a starting material by

(i) removing intact living cells from the blood sample to obtain an acellular fraction and combining said acellular fraction with a process medium, or

(ii) combining the isolated blood sample with a process medium into a first mixture and removing intact living cells from said first mixture;

10 (c) incubating the starting material in an incubation chamber for a first period of time to form an acellular bioabsorbable tissue regeneration matrix; and

(d) isolating the acellular bioabsorbable tissue regeneration matrix;

15 in which said acellular bioabsorbable tissue regeneration matrix has a regenerative effect towards cells which is not present in the starting material, and/or in which said acellular bioabsorbable tissue regeneration matrix has significantly greater regenerative effect towards cells compared to the starting material.

2. A method according to Claim 1, in which the blood sample comprises a blood fraction.

3. A method according to Claim 2, in which the blood fraction comprises a buffy-coat layer.

20 4. A method according to Claim 2, in which the blood fraction comprises a buffy-coat/platelet/plasma layer.

5. A method according to Claim 1, in which the blood sample comprises an anticoagulant.

25 6. A method according to Claim 1, in which the blood sample comprises one or more clots, and the method further comprises solubilizing the one or more clots.

7. A method according to Claim 1, in which the removing step comprises passing the blood sample through a size selection filter.
8. A method according to Claim 7, in which the size selection filter excludes particles larger than about 5 μm .
- 5 9. A method according to Claim 7, in which the size selection filter excludes particles larger than about 1.2 μm .
10. A method according to Claim 7, in which the size selection filter excludes particles larger than about 0.8 μm .
11. A method according to Claim 1, in which the incubation chamber is impermeable to
10 solid or liquid matter.
12. A method according to Claim 11, in which the incubation chamber is permeable to gases generated by evaporation of liquid inside the incubation chamber.
13. A method according to Claim 12, in which the incubation chamber is incubated in a low humidity environment, such that the evaporation rate of liquid inside the incubation
15 chamber is increased.
14. A method according to Claim 1, further comprising adding process medium to the isolated blood sample and/or the acellular fraction and/or the starting material during isolation of the blood sample; prior to the removing step; prior to the combining step; during the combining step; prior to the incubating step; during the incubating step; or any combination
20 thereof.
15. A method according to Claim 1, in which the acellular sample is in the form of a liquid solution or suspension.
16. A method according to Claim 12, in which the evaporation of liquid results in an increase in osmolarity of the liquid solution or suspension over the first period of time.
- 25 17. A method according to Claim 14, in which the process medium comprises a physiological solution.

18. A method according to Claim 1, in which the first period of time is at least 5 days.
19. A method according to Claim 1, in which the first period of time is at least 12 days.
20. A method according to Claim 1, in which the incubating step comprises incubating the acellular sample in a low oxygen environment.
- 5 21. A method according to Claim 20, in which the low oxygen environment comprises an environment with less than about 5% oxygen.
22. A method according to Claim 20, in which the low oxygen environment comprises an environment that substantially lacks oxygen.
23. A method according to Claim 20, in which the first period of time is at least 3 days.
- 10 24. A method according to Claim 20, in which the first period of time is at least 7 days.
25. A method according to Claim 1, further comprising adding one or more therapeutic agents at one or more steps selected from the group consisting of:
 - (i) to the blood sample during the isolation thereof prior to step (a);
 - (ii) to the blood sample prior to step (b);
 - 15 (iii) to the acellular fraction prior to step (c);
 - (iv) to the acellular fraction during step (b);
 - (v) to the starting material after step (b);
 - (vi) to the starting material during step (c);
 - (vii) to the regeneration matrix after step (c);
 - 20 (viii) to the isolated regeneration matrix after (d); and
 - (ix) any combination thereof.
26. A method according to Claim 25, in which the one or more therapeutic agents is selected from the group consisting of: proteins, peptides, drugs, cytokines, extracellular matrix molecules, growth factors, and any combination thereof.

27. A method according to Claim 1, in which the osmolarity of the acellular sample is controlled such that the acellular bioabsorbable tissue regeneration matrix formed during the first period of time is in the form of a three-dimensional matrix.

28. A method according to Claim 1, in which the osmolarity of the acellular sample is controlled such that the acellular bioabsorbable tissue regeneration matrix formed during the first period of time is in the form of a suspension.

29. A method according to Claim 1, further comprising seeding or mixing the acellular bioabsorbable tissue regeneration matrix with cells after formation thereof, in which the cells are added during or after step (d).

30. A method according to Claim 29, in which the cells are selected from the group consisting of: stem cells, progenitor cells, somatic cells, and any combination thereof.

31. A method according to Claim 29, in which the cells are selected from the group consisting of: embryonic stem cells, neural stem cells, neuroprogenitor cells, neurons, glial cells, and combinations thereof.

32. A method according to Claim 1, further comprising seeding or mixing the acellular bioabsorbable tissue regeneration matrix with cells while it is being produced.

33. A method according to Claim 32, in which the cells are cells selected from the group consisting of: stem cells, progenitor cells, somatic cells, and any combination thereof.

34. A method according to Claim 32, in which the cells are cells selected from the group consisting of: embryonic stem cells, neural stem cells, neuroprogenitor cells, neurons, glial cells, and combinations thereof.

35. A method according to Claim 1, in which the acellular bioabsorbable tissue regeneration matrix supports, induces and/or controls an activity selected from the group consisting of: tissue growth, cell growth, gene upregulation, cell inhibition, cell proliferation, cell dedifferentiation, cell differentiation, cell transdifferentiation, neurite outgrowth, one or more natural wound healing processes, and any combination thereof.

36. An isolated acellular bioabsorbable tissue regeneration matrix comprising: a proteinaceous core having a protein content of at least 1%; in which the structure of the acellular bioabsorbable tissue regeneration matrix is characterized by spherical bodies with a diameter of approximately at least 100 nm; and further in which the acellular bioabsorbable tissue regeneration matrix lacks substantial metabolic activity; and further in which the acellular bioabsorbable tissue regeneration matrix is capable of initiating more tissue regeneration in a subject with tissue damage, increasing tissue regeneration in a subject with tissue damage, or both.

37. An isolated acellular bioabsorbable tissue regeneration matrix according to Claim 36, further comprising one or more proteins selected from the group consisting of: transferrin, serum albumin, serum albumin precursor, complement component 3, chains A-D hemoglobin, IgM, IgG1, medullasin inhibitor 2, carbonic anhydrase, CA1 protein, and combinations thereof.

38. An isolated acellular bioabsorbable tissue regeneration matrix according to Claim 36, in which the spherical bodies are recognized by CD56 antibodies.

39. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, in which the spherical bodies have a diameter of about 1-2 μm .

40. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, in which the spherical bodies have a diameter of about 2-4 μm .

41. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, further comprising a therapeutic agent.

42. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the therapeutic agent is distributed heterogeneously within the acellular bioabsorbable tissue regeneration matrix.

43. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the therapeutic agent is distributed homogeneously within the acellular bioabsorbable tissue regeneration matrix.

44. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the acellular bioabsorbable tissue regeneration matrix comprises two or more therapeutic agents.

45. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the therapeutic agent is selected from the group consisting of: proteins, peptides, drugs, cytokines, extracellular matrix molecules, growth factors, and combinations thereof.

46. An acellular bioabsorbable tissue regeneration matrix according to Claim 45, in which the acellular bioabsorbable tissue regeneration matrix increases the magnitude of one or more beneficial effects of the therapeutic agent.

47. An acellular bioabsorbable tissue regeneration matrix according to Claim 45, in which the acellular bioabsorbable tissue regeneration matrix prolongs one or more beneficial effects of the therapeutic agent by slowly releasing the therapeutic agent over time in the subject.

48. An acellular bioabsorbable tissue regeneration matrix according to Claim 45, in which the acellular bioabsorbable tissue regeneration matrix protects one or more beneficial effects of the therapeutic agent from substantially decreasing over time.

49. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, further comprising cells.

50. An acellular bioabsorbable tissue regeneration matrix according to Claim 49, in which the cells are selected from the group consisting of: stem cells, progenitor cells, somatic cells, and combinations thereof.

51. An acellular bioabsorbable tissue regeneration matrix according to Claim 49, in which the cells are cells selected from the group consisting of: embryonic stem cells, neural stem cells, neuroprogenitor cells, neurons, glial cells, and combinations thereof.

52. A method according to Claim 1, in which the removing of intact cells is effected by freezing, filtration and/or centrifugation.

53. An acellular bioabsorbable tissue regeneration matrix generated according to a method according to any one of Claims 1-35 or 52, in which the acellular regeneration matrix;

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- (a) comprises aggregates of spherical structures having approximately 100 nm to 4 microns in diameter,
- (b) is self-assembled;
- (c) comprises a proteinaceous core having a majority of protein content derived from blood;
- (d) lacks substantial metabolic activity; or
- (e) any combination of (a) to (d).

CLAIMS

1. A method of producing an acellular bioabsorbable tissue regeneration matrix, the method comprising:

(a) providing an isolated blood or bone marrow sample;

5 (b) obtaining a starting material by

(i) lysing or removing intact living cells from the blood or bone marrow sample to obtain an acellular fraction and combining said acellular fraction with a process medium, or

10 (ii) combining the isolated blood or bone marrow sample with a process medium into a first mixture and lysing or removing intact living cells from said first mixture;

(c) incubating the starting material in an incubation chamber for a first period of time to form an acellular bioabsorbable tissue regeneration matrix; and

(d) isolating the acellular bioabsorbable tissue regeneration matrix;

15 in which said acellular bioabsorbable tissue regeneration matrix has a regenerative effect towards cells which is not present in the starting material, and/or in which said acellular bioabsorbable tissue regeneration matrix has significantly greater regenerative effect towards cells compared to the starting material.

20 2. A method according to Claim 1, in which the blood or bone marrow sample comprises a blood or bone marrow fraction.

3. A method according to Claim 2, in which the blood or bone marrow fraction comprises a buffy-coat layer.

4. A method according to Claim 2, in which the blood or bone marrow fraction comprises a buffy-coat/platelet/plasma layer.

25 5. A method according to Claim 1, in which the blood or bone marrow sample comprises an anticoagulant.

6. A method according to Claim 1, in which the blood or bone marrow sample comprises one or more clots, and the method further comprises solubilizing the one or more clots.
7. A method according to Claim 1, in which the removing step comprises passing the blood or bone marrow sample through a size selection filter.
- 5 8. A method according to Claim 7, in which the size selection filter excludes particles larger than about 5 μm .
9. A method according to Claim 7, in which the size selection filter excludes particles larger than about 1.2 μm .
- 10 10. A method according to Claim 7, in which the size selection filter excludes particles larger than about 0.8 μm .
11. A method according to Claim 1, in which the incubation chamber is impermeable to solid or liquid matter.
12. A method according to Claim 11, in which the incubation chamber is permeable to gases generated by evaporation of liquid inside the incubation chamber.
- 15 13. A method according to Claim 12, in which the incubation chamber is incubated in a low humidity environment, such that the evaporation rate of liquid inside the incubation chamber is increased.
14. A method according to Claim 1, further comprising adding process medium to the isolated blood or bone marrow sample and/or the acellular fraction and/or the starting material
20 during isolation of the blood or bone marrow sample; prior to the lysing or removing step; prior to the combining step; during the combining step; prior to the incubating step; during the incubating step; or any combination thereof.
15. A method according to Claim 1, in which the acellular sample is in the form of a liquid solution or suspension.
- 25 16. A method according to Claim 12, in which the evaporation of liquid results in an increase in osmolarity of the liquid solution or suspension over the first period of time.

17. A method according to Claim 14, in which the process medium comprises a physiological solution.
18. A method according to Claim 1, in which the first period of time is at least 5 days.
19. A method according to Claim 1, in which the first period of time is at least 12 days.
- 5 20. A method according to Claim 1, in which the incubating step comprises incubating the acellular sample in a low oxygen environment.
21. A method according to Claim 20, in which the low oxygen environment comprises an environment with less than about 5% oxygen.
22. A method according to Claim 20, in which the low oxygen environment comprises an
10 environment that substantially lacks oxygen.
23. A method according to Claim 20, in which the first period of time is at least 3 days.
24. A method according to Claim 20, in which the first period of time is at least 7 days.
25. A method according to Claim 1, further comprising adding one or more therapeutic agents at one or more steps selected from the group consisting of:
 - 15 (i) to the blood or bone marrow sample during the isolation thereof prior to step (a);
 - (ii) to the blood or bone marrow sample prior to step (b);
 - (iii) to the acellular fraction prior to step (c);
 - (iv) to the acellular fraction during step (b);
 - 20 (v) to the starting material after step (b);
 - (vi) to the starting material during step (c);
 - (vii) to the regeneration matrix after step (c);
 - (viii) to the isolated regeneration matrix after (d); and
 - (ix) any combination thereof.

26. A method according to Claim 25, in which the one or more therapeutic agents is selected from the group consisting of: proteins, peptides, drugs, cytokines, extracellular matrix molecules, growth factors, and any combination thereof.
27. A method according to Claim 1, in which the osmolarity of the acellular sample is controlled such that the acellular bioabsorbable tissue regeneration matrix formed during the first period of time is in the form of a three-dimensional matrix.
28. A method according to Claim 1, in which the osmolarity of the acellular sample is controlled such that the acellular bioabsorbable tissue regeneration matrix formed during the first period of time is in the form of a suspension.
29. A method according to Claim 1, further comprising seeding or mixing the acellular bioabsorbable tissue regeneration matrix with cells after formation thereof, in which the cells are added during or after step (d).
30. A method according to Claim 29, in which the cells are selected from the group consisting of: stem cells, progenitor cells, somatic cells, and any combination thereof.
31. A method according to Claim 29, in which the cells are selected from the group consisting of: embryonic stem cells, neural stem cells, neuroprogenitor cells, neurons, glial cells, and combinations thereof.
32. A method according to Claim 1, further comprising seeding or mixing the acellular bioabsorbable tissue regeneration matrix with cells while it is being produced.
33. A method according to Claim 32, in which the cells are cells selected from the group consisting of: stem cells, progenitor cells, somatic cells, and any combination thereof.
34. A method according to Claim 32, in which the cells are cells selected from the group consisting of: embryonic stem cells, neural stem cells, neuroprogenitor cells, neurons, glial cells, and combinations thereof.
35. A method according to Claim 1, in which the acellular bioabsorbable tissue regeneration matrix supports, induces and/or controls an activity selected from the group consisting of: tissue growth, cell growth, gene upregulation, cell inhibition, cell proliferation,

cell dedifferentiation, cell differentiation, cell transdifferentiation, neurite outgrowth, one or more natural wound healing processes, and any combination thereof.

36. An isolated acellular bioabsorbable tissue regeneration matrix derived from blood or bone marrow comprising: a heterogeneous mixture of proteins and salts, in which the overall protein content of the matrix is at least 1%, in which the matrix is characterized by aggregates of spherical structures with a diameter of approximately at least 100 nm; in which the acellular bioabsorbable tissue regeneration matrix lacks substantial metabolic activity as compared to that initially present in blood or bone marrow; and further in which the acellular bioabsorbable tissue regeneration matrix is capable of initiating tissue regeneration in a subject with tissue damage, or increasing tissue regeneration in a subject with tissue damage compared with untreated tissue, or both.

37. An isolated acellular bioabsorbable tissue regeneration matrix according to Claim 36, further comprising one or more proteins selected from the group consisting of: transferrin, serum albumin, serum albumin precursor, complement component 3, chains A-D hemoglobin, IgM, IgG1, medullasin inhibitor 2, carbonic anhydrase, CA1 protein, and combinations thereof.

38. An isolated acellular bioabsorbable tissue regeneration matrix according to Claim 36, in which the spherical bodies are recognized by CD56 antibodies.

39. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, in which the spherical structures have a diameter of about 1-2 μm .

40. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, in which the spherical structures have a diameter of about 2-4 μm .

41. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, further comprising a therapeutic agent.

42. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the therapeutic agent is distributed heterogeneously within the acellular bioabsorbable tissue regeneration matrix.

43. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the therapeutic agent is distributed homogeneously within the acellular bioabsorbable tissue regeneration matrix.

44. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the acellular bioabsorbable tissue regeneration matrix comprises two or more therapeutic agents.

45. An acellular bioabsorbable tissue regeneration matrix according to Claim 41, in which the therapeutic agent is selected from the group consisting of: proteins, peptides, drugs, cytokines, extracellular matrix molecules, growth factors, and combinations thereof.

46. An acellular bioabsorbable tissue regeneration matrix according to Claim 45, in which the acellular bioabsorbable tissue regeneration matrix increases the magnitude of one or more beneficial effects of the therapeutic agent.

47. An acellular bioabsorbable tissue regeneration matrix according to Claim 45, in which the acellular bioabsorbable tissue regeneration matrix prolongs one or more beneficial effects of the therapeutic agent by slowly releasing the therapeutic agent over time in the subject.

48. An acellular bioabsorbable tissue regeneration matrix according to Claim 45, in which the acellular bioabsorbable tissue regeneration matrix protects one or more beneficial effects of the therapeutic agent from substantially decreasing over time.

49. An acellular bioabsorbable tissue regeneration matrix according to Claim 36, further comprising exogenous cells that have been added to the matrix.

50. An acellular bioabsorbable tissue regeneration matrix according to Claim 49, in which the cells are selected from the group consisting of: stem cells, progenitor cells, somatic cells, and combinations thereof.

51. An acellular bioabsorbable tissue regeneration matrix according to Claim 49, in which the cells are cells selected from the group consisting of: embryonic stem cells, neural stem cells, neuroprogenitor cells, neurons, glial cells, and combinations thereof.

52. A method according to Claim 1, in which the removing of intact cells is effected by freezing, filtration and/or centrifugation.

53. An acellular bioabsorbable tissue regeneration matrix generated according to a method according to any one of Claims 1 to 35 or 52, in which the acellular regeneration matrix:

- 5 (a) comprises aggregates of spherical structures with a diameter of approximately at least 100 nm;
- (b) is self-assembled;
- (c) comprises a proteinaceous core having a majority of protein content derived from blood or bone marrow;
- 10 (d) lacks substantial metabolic activity; or
- (e) any combination of (a) to (d).