CLAIMS

1. A method for producing extracellular vesicles (EVs) from stem cells, the method comprising the steps of:

a) providing a population of stem cells cultured on at least one three-dimensional porous scaffold;

b) providing conditions for three-dimensional multi-layer expansion of the stem cells cultured on the at least one three-dimensional porous scaffold;

c) providing shear stress stimulations at above about 0.5 dyne/cm^2 to said population of stem cells, wherein the population of stem cells secretes extracellular vesicles into a medium;

d) collecting the medium; and

e) isolating the secreted EVs dispersed therein.

2. The method according to claim 1, wherein at step (c) the shear stress stimulations are in the range of ~~about~~ 0.5 to ~~about~~ 100 dyne/cm^2.

3. The method according to claim 2, wherein at step (c) the shear stress stimulations are in the range of ~~about~~ 5 to ~~about~~ 30 dyne/cm^2.

4. The method according to any one of claims 1-3, wherein the stem cells are seeded and cultured on the least one three-dimensional porous scaffold prior to providing shear stress stimulations thereto.

5. The method according to any one of claims 1-4, wherein step (a) comprises providing a system configured to deliver a medium through a population of stem cells, the system comprising: a flow chamber comprising an inlet port, an outlet port, and at least one flow chamber wall defining an internal chamber; an oxygenator; a medium reservoir comprising the medium; and a pump, wherein the flow chamber, the oxygenator, the medium reservoir and the pump are in fluid communication with each other.

6. The method according to claim 5, wherein at least one of the oxygenator and the flow chamber are disposed within the medium reservoir.

7. The method according to claims 5 and 6, wherein step (b) is performed within the flow chamber, wherein the at least one three-dimensional porous scaffold is disposed within the flow chamber.

8. The method according to any one of claims claim 5-7, wherein step (c) comprises flowing the medium into the flow chamber, wherein the medium enters the flow chamber through the inlet port at a predetermined flow rate, flows through the at

least one three-dimensional porous scaffold, and exits through the outlet port, thereby providing shear stress stimulations to the population of stem cells cultured thereon.

9. The method according to claim 8, wherein the predetermined flow rate is in the range of ~~about~~ 0.01 to ~~about~~ 100 ml/min.

10. The method according to claim 9, wherein the predetermined flow rate is in the range of ~~about~~ 0.1 to ~~about~~ 10 ml/min.

11. The method according to any one of claims 8-10, wherein at step (c) the medium flows through the at least one three-dimensional porous scaffold at a flow velocity in the range of ~~about~~ 0.1 to ~~about~~ 100 cm/min.

12. The method according to claim 11, wherein the flow velocity in the range of ~~about~~ 0.1 to ~~about~~ 5 cm/min.

13. The method according to any one of claims claim 5-7, wherein step (c) comprises moving the at least one three-dimensional porous scaffold within the flow chamber, thereby providing shear stress stimulations to the population of stem cells cultured thereon, wherein the movement of the at least one three-dimensional porous scaffold within the flow chamber is selected from agitating, vibrating, rotating, waving, or tilting the at least one three-dimensional porous scaffold.

14. The method according to any one of claims 1-13, wherein step (c) is performed for ~~about~~ 1 hour to ~~about~~ 30 days.

15. The method according to claim 14, wherein step (c) is performed for about 48 hours.

16. The method according to any one of claims 5-15, wherein the flow chamber is a reactor selected from the group consisting of: laminar flow reactor (LFR), plug flow reactor (PFR), continuous stirred-tank reactor (CSTR), batch reactor, heterogenous catalytic reactor, fed-batch bioreactor, perfusion bioreactor, fix-bed bioreactor, packed bed bioreactor, wave bioreactor, air lift bioreactor, and vibrating bed.

17. The method according to any one of claims 1-16, wherein the at least one three-dimensional porous scaffold comprises at least one material selected from the group consisting of: polyester, polypropylene, polylactic acid (PLA), Poly-L-lactic acid (PLLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), cellulose, silk, glass, and natural and synthetic hydrogels selected from: gelatin, collagen, fibrin, PEG, alginate, and chitosan.

18. The method according to any one of claims 1-17, wherein the at least one three-dimensional porous scaffold comprises a plurality of three-dimensional porous scaffolds.

19. The method according to any one of claims 1-18, wherein the extracellular vesicles are selected from the group consisting of: exosomes, microvesicles, apoptotic bodies, and ectosomes.

20. The method according to any one of claims 1-19, wherein the stem cells are naïve or engineered human stem cells.

21. The method according to any one of claims 20, wherein the stem cells are selected from the group consisting of: adult stem cells, embryonic stem cells (ESCs), induced pluripotent stem cells, cord blood stem cells and amniotic fluid stem cells.

22. The method according to claim 21, wherein the adult stem cells are selected from the group consisting of: neural stem cells, skin stem cells, epithelial stem cells, skeleton muscle satellite cells, mesenchymal stem cells, adipose-derived stem cells, endothelial stem cells, dental pulp stem cells (DPSCs), hematopoietic stem cells and placenta derived stem cells.

23. The method according to any one of claims 1-22, wherein the system further comprises at least one or more sensors for measuring in the medium at least one parameter selected from the group consisting of pressure, flow rate, temperature, pH, dissolved oxygen, concentration of medium components, and extracellular vesicles quantity.

24. The method according to claim 23, wherein system further comprises a control unit in operative communication with the at least one or more sensors, configured to receive measurements of the at least one parameter and adjust the at least one parameter based on the measurements.

25. The method according to any one of claims 1-24, wherein the flow chamber is not a hollow fiber bioreactor (HFBR), and the system does not comprise a hollow fiber bioreactor.

~~26. A system configured to deliver a medium through a population of cultured stem cells, the system comprising: a flow chamber comprising: an inlet port, an outlet port, and at least one flow chamber wall defining an internal chamber; an oxygenator; a medium reservoir comprising a medium; and a pump, wherein the flow chamber, the oxygenator, the medium reservoir and the pump are in fluid communication with each other.~~

~~27. Extracellular vesicles produced according to the method according to any one of claims 1-25.~~

~~28. A composition comprising the extracellular vesicles according to claim 27.~~

~~29. The extracellular vesicles according to claim 27 or the composition according to claim 28, for use in the prevention or treatment of a disease or disorder, selected from the group consisting of: inflammatory diseases; autoimmune diseases; blood vessel diseases; cardiac diseases; respiratory system diseases; skeletal system diseases; gastrointestinal tract diseases; kidney disease; urinary tract diseases; skin diseases; ageing associated diseases; peripheral nerve and skeletal muscle diseases; diseases of the central nervous system; eye diseases; diseases of the endocrine system; cancer; diabetes; and dental and oral diseases.~~

~~30. A method of prevention or treatment of a disease or disorder, comprising administering to a subject in need thereof a composition according to claim 28, wherein the disease or disorder is selected from the group consisting of: inflammatory diseases; autoimmune diseases; blood vessel diseases; cardiac diseases; respiratory system diseases; skeletal system diseases; gastrointestinal tract diseases; kidney disease; urinary tract diseases; skin diseases; ageing associated diseases; peripheral nerve and skeletal muscle diseases; diseases of the central nervous system; eye diseases; diseases of the endocrine system; cancer; diabetes; and dental and oral diseases.~~