CLAIMS

1. A method for producing extracellular vesicles (EVs) from an engineered tissue containing stem cells therein, the method comprising the steps of:
    a. embedding a population of stem cells in at least one three-dimensional porous scaffold;
    b. culturing the stem cells on and/or within the at least one three-dimensional porous scaffold for at least 6 days, to expand the scaffold and form at least one connected stem cell multi-layer engineered tissue containing stem cells and EVs therein, wherein the cultured stem cells adhere to the scaffold and/or to each other and secrete EVs;
    c. providing shear stress stimulations in the range of 5 to 30 dyne/cm^2 to said at least one engineered tissue containing the population of stem cells and EVs, wherein the population of stem cells secretes the EVs into a medium, and wherein the shear stress stimulations induce physiological changes in the cells that result in enhanced secretion of EVs compared to an engineered tissue that was not exposed to shear stress simulations;
    d. collecting the medium containing the secreted EVs; and
    e. isolating the secreted EVs from the medium.

2. The method according to claim 1, wherein the stem cells are cultured on the at least one three-dimensional porous scaffold for at least 9 days to form the engineered tissue containing stem cells and EVs therein prior to providing shear stress stimulations thereto.
3. The method according to claim 1, wherein step (a) further comprises providing a system configured to deliver the medium through the at least one engineered tissue containing the population of stem cells and EVs therein, 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.
4. The method according to claim 3, wherein at least one of the oxygenator and the flow chamber are disposed within the medium reservoir.

5. The method according to claim 3, wherein step (b) is performed within the flow chamber, wherein the at least one engineered tissue is disposed within the flow chamber.
6. The method according to claim 3, wherein step (c) further 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 engineered tissue, and exits through the outlet port, thereby applying shear stress stimulations on the population of stem cells and EVs in the engineered tissue.
7. The method according to claim 6, wherein the predetermined flow rate is in the range of 0.1 to 1.5 ml/min.
8. The method according to claim 6, wherein step (c) further comprises moving the at least one engineered tissue within the flow chamber, thereby providing shear stress stimulations to the population of stem cells and EVs in the engineered tissue, wherein the movement of the at least one engineered tissue within the flow chamber is selected from agitating, vibrating, rotating, waving, or tilting.
9. The method according to claim 6, wherein step (c) is performed for 1 hour to 30 days.
10. The method according to claim 1, wherein the at least one engineered tissue 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.
11. The method according to claim 1, wherein the extracellular vesicles are selected from the group consisting of: exosomes, microvesicles, apoptotic bodies, and ectosomes.
12. The method according to claim 1, wherein the stem cells are naive or engineered human stem cells.
13. The method according to claim 12, 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.

14. The method according to claim 13, 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.
15. The method according to claim 11, wherein the extracellular vesicles comprise at least one upregulated protein selected from the group consisting of: RhoG, ITGAV, CAPZA2, CKAP5, CDH13, ARPC2, ARPC4, MYHII, TUBAIB, TUBB, TUBB2B, TUBB2A, and combinations thereof.
16. The method according to claim 11, wherein the extracellular vesicles which were isolated from the medium in step (e) comprise at least one marker selected from CD9, CD63, and CD81, and further comprise at least one upregulated protein selected from the group consisting of: RhoG, ITGAV, CAPZA2, CKAP5, CDH13, ARPC2, ARPC4, MYHII, TUBAIB, TUBB, TUBB2B, TUBB2A, and combinations thereof.
17. A method for producing extracellular vesicles (EVs) from an engineered tissue containing stem cells therein, the method comprising the steps of:
    a. embedding a population of stem cells in at least one three-dimensional porous scaffold;
    b. culturing the stem cells on and/or within the at least one three-dimensional porous scaffold for at least 6 days, to expand the scaffold and form at least one connected stem cell multi-layer engineered tissue containing stem cells and EVs therein, wherein the cultured stem cells adhere to the scaffold and/or to each other and secrete EVs;
    c. delivering a medium to the at least one connected stem cell multi-layer engineered tissue which is disposed in a chamber with the medium delivered at a flow rate in the range of 0.1 to 1.5 ml/min, thereby providing shear stress stimulations in the range of 5 to 30 dyne/cm^2 to said at least one engineered tissue containing the population of stem cells and EVs, wherein the population of stem cells secretes the EVs into the medium, and wherein the shear stress stimulations induce physiological changes in the cells that result in enhanced secretion of EVs compared to an engineered tissue that was not exposed to shear stress simulations;

d. collecting the medium containing the secreted EVs; and
e. isolating the secreted EVs from the medium.

18. A method for producing extracellular vesicles (EVs) from an engineered tissue containing stem cells therein, the method comprising the steps of:
    a. embedding a population of stem cells in at least one three-dimensional porous scaffold;
    b. culturing the stem cells on the at least one three-dimensional porous scaffold for at least 6 days, to expand the scaffold and form at least one connected stem cell multi-layer engineered tissue containing stem cells and EVs therein, wherein the cultured stem cells adhere to the scaffold and/or to each other while secreting EVs;
    c. providing shear stress stimulations in the range of 5 to 30 dyne/cm^2 to said at least one engineered tissue containing the population of stem cells and EVs, wherein the population of stem cells secretes the EVs into the medium, and wherein the shear stress stimulations induce physiological changes in the cells that result in enhanced secretion of EVs compared to an engineered tissue that was not exposed to shear stress simulations;
    d. collecting the medium containing the secreted EVs; and
    e. isolating the secreted EVs from the medium.

    wherein step (c) is performed within a chamber with the at least one engineered tissue and medium disposed therein, by moving the at least one engineered tissue within the chamber, thereby providing the shear stress stimulations to the population of stem cells and EVs in the engineered tissue, wherein the movement of the at least one engineered tissue in the chamber is selected from agitating, vibrating, rotating, waving, or tilting.