~~We claim~~CLAIMS:

1. A method of preparing a Master Cell Bank composition comprising Mesenchymal stem cells, Fetal Bovine Serum (FBS) and Dimethyl Sulphoxide (DMSO), said method comprising acts of:
    a. obtaining and diluting bone marrow in culture media comprising Dulbecco's Modified Eagle Medium-KnockOut [DMEM-KO], Fetal Bovine Serum [FBS], Glutamine and Pen-Strep and centrifuging the diluted bone marrow to obtain a pellet and re-diluting the pellet with the culture media;
    b. adding density gradient solution to the re-diluted pellet and centrifuging to obtain a buffy layer at interface;
    c. to the buffy layer, adding equal volume of the culture media and centrifuging to obtain a pellet comprising Mononuclear cells (MNC), followed by suspending the pellet in the culture media; seeding 40—-50 million Mononuclear cells (MNC) and culturing with media change to achieve about 80% to 85% confluency;
    d. aspirating the culture media and washing the confluent cells with Dulbecco's Phosphate Buffered Saline (DPBS), thereafter treating the cells with 0.25% trypsin/EDTA to obtain trypsinized cells-;
    e. neutralizing the trypsinized cells with neutralization media and centrifuging the cells to obtain a pellet comprising Mesenchymal Stem Cells (MSC), followed by suspending the pellet in the culture media;
    f. seeding the Mesenchymal Stem Cells at a seeding density of 6666 cells/cm^2 and culturing with media change to achieve about 80% to 85% confluency;
    g. harvesting the cells of step f) by aspirating the culture media, washing the confluent cells with DPBS, trypsinizing the cells, neutralizing the trypsinized cells with Neutralization media and centrifuging to obtain pellet comprising Mesenchymal Stem Cells; and
    h. suspending the Mesenchymal Stem Cells in freezing media comprising about 85% to 95% FBS and about 5% to 15% DMSO to obtain the Master Cell Bank composition.

2. The method as claimed in claim 1, wherein the culturing is carried out in a 5% CO_2 incubator at a temperature of about 37°C.

3. The method as claimed in claim 1, wherein the confluency is obtained by replenishing the culture media after 7 days to 8 days.

4. The method as claimed in claim 1, wherein the neutralization media comprises Dulbecco's Modified Eagle Medium-KnockOut [DMEM KO], about 10% Fetal Bovine Serum [FBS] and about 0.5% Pen-Strep.

5. The method as claimed in claim 1, wherein the cells in step d) are treated with 0.25 % ~~T~~trypsin/EDTA in a 37°C incubator for 2 minutes to 3 minutes.

6. A method of preparing a Working Cell Bank composition comprising Mesenchymal stem cells, Fetal Bovine Serum (FBS) and Dimethyl Sulphoxide (DMSO), said method comprising acts of:

   a. preparing Master Cell Bank compositions ~~according to claim 1~~ from bone marrow cells obtained from different donors according to claim 1, wherein each Master Cell Bank composition is obtained from bone marrow cells of a single donor-;
   b. obtaining cell pellet from the Master Cell Bank composition, followed by suspending the cell pellet from each Master Cell Bank composition in culture media and pooling the cells from each of the Master Cell Bank compositions in equal proportion to obtain pooled cells;
   c. culturing the pooled cells with the culture media to achieve about 80% to 85% confluency and re-performing step (d) of claim 1;
   d. neutralizing the trypsinized cells with the culture media, followed by centrifuging the cells to obtain a pellet and suspending the pellet in the culture media to obtain cultured cells; and
   e. re-centrifuging the cultured cells and freezing the cell pellet with freezing media comprising about 85 to 95% FBS and about 5 to 15% DMSO to obtain the Working Cell Bank composition.

7. The method as claimed in claim 6, wherein the Master Cell Bank composition comprises Mesenchymal stem cells ranging from about 1 million cells to 3 million cells per ml, Fetal Bovine Serum (FBS) ranging from about 85% to 95% and Dimethyl Sulphoxide (DMSO) ranging from about 5% to 15%.

8. The method as claimed in claim 6, wherein the obtaining of the cell pellet is by thawing stem cells, neutralizing freezing media with ~~complete~~ culture media and centrifuging at about 1200 rpm to 1500 rpm for time duration of about 10 to 20 minutes to obtain cell pellet.

9. A method of preparing a composition comprising Mesenchymal Stem Cells, ~~Plasmalyte-A~~Multiple Electrolytes Injection, Type I, USP, Human Serum Albumin (HSA), Dimethyl Sulphoxide (DMSO), optionally along with pharmaceutically acceptable additives, said method comprising acts of:
   a. preparing a Working Cell Bank composition by pooling Mesenchymal Stem Cells from multiple Master Cell Bank compositions, according to claim 6,
   b. obtaining pooled Mesenchymal Stem Cells from the composition, followed by culturing the cells with culture media to achieve about 80% to 85% confluency, and re-performing step (d) of claim 1;
   c. neutralizing the trypsinized cells with the culture media and thereafter centrifuging the cells to obtain a cell pellet; washing the cell pellet in DPBS followed by re-centrifuging to obtain a cell pellet;
   d. suspending the cell pellet in the ~~complete~~ culture media, culturing the cells in the ~~complete~~ culture media to achieve about 80% to 85% confluency;
   e. harvesting of the confluent cells by washing the cells with the DPBS; treating the washed cells with 0.25% trypsin and neutralizing the trypsinized cells with the ~~complete~~ culture media;
   f. centrifuging the neutralized cell suspension and washing the centrifuged cells with the DPBS followed by re-centrifuging to obtain a pellet; and
   g. freezing the pellet with the ~~Plasmalyte-A~~ Multiple Electrolytes Injection, Type I, USP, the DMSO and the Human Serum Albumin; and optionally adding pharmaceutically acceptable additive to obtain the composition.

10. The method as claimed in claim 9, wherein the Working Cell Bank composition has Mesenchymal Stem Cells ranging from about 1 million cells to 3 million cells per ml, Fetal Bovine Serum (FBS) ranging from about 85% to 95% and Dimethyl Sulphoxide (DMSO) ranging from about 5% to 15%.

11. The method as claimed in claims 1, 6 and 9, wherein the centrifuging is carried out at about 1200 rpm to 1500 rpm, at temperature ranging from about 20°C to 25°C, for time duration ranging from about 10 minutes to 20 minutes.

12. The method as claimed in claims 6 and 9, wherein the culturing is done in a 5% CO_2 incubator at a temperature of about 37°C; and wherein the confluency is obtained by replenishing the culture media after about 7 days to 8 days.

13. The method as claimed in claims 6 and 9, wherein the culture media comprises Dulbecco's Modified Eagle Medium-KnockOut [DMEM-KO] at a concentration ranging from about 85% to 95%, Fetal Bovine Serum [FBS] at a concentration ranging from about 5% to 15%, Glutamine at a concentration ranging from about 0.5% to 2%; Pen-Strep at a concentration ranging from about 0.1% to 1%; and basic Fibroblast Growth Factor [bFGF] at a concentration ranging from about 0.5 ng/ml to 5 ng/ml.

14. A composition comprising pooled Mesenchymal Stem Cells, Human Serum Albumin (HSA), ~~Plasmalyte A~~ Multiple Electrolytes Injection, Type I, USP and Dimethyl Sulphoxide (DMSO), optionally along with pharmaceutically acceptable additives.

15. A Master Cell Bank Composition prepared by the method as claimed in claim 1, wherein the composition comprises ~~comprising~~ Mesenchymal Stem cells~~,~~ having concentration ranging from about 1 million to 3 million cells per ml, Fetal Bovine Serum (FBS) having concentration ranging from about 85% to 95% and Dimethyl Sulphoxide (DMSO) having concentration ranging from about 5% to 15%.

16. A Working Cell Bank Composition comprising pooled Mesenchymal Stem Cells, Fetal Bovine Serum (FBS) and Dimethyl Sulphoxide (DMSO).

17. The composition as claimed in claim 14, wherein the Mesenchymal Stem Cells have concentration ranging from about 25 million to 200 million cells per 15ml; the Human Serum Albumin has concentration ranging from about 1% to 6%; the ~~Plasmalyte A~~ Multiple Electrolytes Injection, Type I, USP has concentration ranging from about 80% to 90%; and the Dimethyl Sulphoxide has concentration ranging from about 5% to 10% in each cryo-preserved vial.

18. The composition as claimed in claim 16, wherein the Mesenchymal Stem Cells have concentration ranging from about 1 million to 3 million cells per ml; the Fetal Bovine Serum has concentration ranging from about 85% to 95%; and the Dimethyl Sulphoxide has concentration ranging from about 5% to 15% in each cryo-preserved vial.

19. The composition as claimed in claim 14~~, wherein the pharmaceutically acceptable additive is Plasmalyte A.~~ and

~~20.~~ ~~T~~the method as claimed in claim 9, wherein the pharmaceutically acceptable additive is ~~Plasmalyte A~~ Multiple Electrolytes Injection, Type I, USP.

~~21. A Kit comprising compositions of claims 14, 15 or 16, and an instructions manual.~~

~~22.~~20. The method as claimed in claim 1, wherein the culture media comprises Dulbecco's Modified Eagle Medium-KnockOut [DMEM-KO] at a concentration ranging from about 85% to 95%, Fetal Bovine Serum [FBS] at a concentration ranging from about 5% to 15%, Glutamine at a concentration ranging from about 0.5% to 2%; and Pen-Strep at a concentration ranging from about 0.1% to 1%.