CLAIMS

Claim 1

A method for preparing mesenchymal stem cells from human pluripotent stem cells, the method comprising the following steps:

(a) culturing human pluripotent stem cells in a serum-free pluripotent stem cell culture medium without xeno feeder cells to obtain a colony of human pluripotent stem cells and isolating human pluripotent stem cells from the colony;

(b) forming single spheroidal embryoid bodies by suspending the isolated pluripotent stem cells in an embryoid body formation medium, and then culturing the isolated pluripotent stem cells such that pluripotent stem cells are aggregated by hanging drop culture, or culture using a V-shape tube, a round shape 96 well plate, or a conical tube;

(c) forming mature embryoid bodies by suspension-culturing the embryoid bodies in an embryoid body maturation medium;

(d) inducing differentiation into mesenchymal stem cells by adherently culturing the embryoid bodies in a xeno-free and serum-free mesenchymal stem cell culture medium; and

(e) proliferating and culturing the differentiated mesenchymal stem cells in a xeno-free and serum-free mesenchymal stem cell culture medium while maintaining the identity of mesenchymal stem cells.

Claim 2

The method of claim 1, wherein the human pluripotent stem cells are embryonic stem cells or induced pluripotent stem cells.

Claim 3

The method of claim 1, wherein the human pluripotent stem cells are in an undifferentiated state.

Claim 4

The method of claim 1, wherein the human pluripotent stem cells in Step (a) are cultured in a culture vessel coated with a xeno-free material selected from the group consisting of human-derived vitronectin, human-derived collagen, human-derived laminin, animal component-free Matrigel and heparan sulfate proteoglycan.

Claim 5

The method of claim 1, wherein the serum-free medium for culturing the human pluripotent stem cells in Step (a) is TeSR-Essential 8 (TeSR-E8) medium, TeSR-2 medium or StemMACS iPS-Brew XF, human medium.

Claim 6

The method of claim 1, wherein the embryoid body formation medium in Step (b) is an Aggrewell™ EB formation medium, Gibco® Essential 6™ medium, CTS™ Essential 6™ medium, or TeSR™-E6 medium.

Claim 7

The method of claim 1, wherein the culture in Step (b) is performed for 18 hours to 30 hours.

Claim 8

The method of claim 1, wherein the embryoid body maturation medium in Step (c) is a basic medium comprising KnockOut™ serum replacement (KSR), non-essential amino acids (NEAA) and β-mercaptoethanol.

Claim 9

The method of claim 8, wherein the basic medium in Step (c) is DMEM/F12, alpha MEM, Ham's F12 media or DMEM.

Claim 10

The method of claim 1, wherein the suspension culture in Step (c) is performed for 10 to 18 days.

Claim 11

The method of claim 1, wherein the mature embryoid bodies obtained in Step (c) have an average size of 350 to 450 μm.

Claim 12

The method of claim 1, wherein the mesenchymal stem cell culture medium in Steps (d) and (e) is a xeno-free and serum-free medium comprising L-glutamine.

Claim 13

The method of claim 12, wherein the xeno-free and serum-free medium is a Stempro® MSC SFM xeno-free medium, a PRIME-XV™ MSC expansion XSFM medium, a human mesenchymal-XF™ expansion medium, an MSC Nutristem® XF medium, a StemMACS® MSC expansion media kit XF, human medium.

Claim 14

The method of claim 1, wherein the induction of differentiation into mesenchymal stem cells in Step (d) is performed for 12 to 20 days.

Claim 15

The method of claim 1, wherein the mesenchymal stem cells in Step (e) are mesenchymal stem cells possessing multipotency capable of differentiating into cells

selected from the group consisting of adipocytes, osteocytes, chondrocytes, myocytes, nerve cells and cardiomyocytes.

Claim 16

The method of claim 1, wherein the mesenchymal stem cells obtained in Step (e) are mesenchymal stem cells expressing CD29(+), CD44(+), CD73(+) and CD105(+) cell surface markers.

Claim 17

The method of claim 16, wherein the expression of the cell surface markers is maintained at 90% or more in mesenchymal stem cells of 20 or more passages.

Claim 18

The method of claim 1, wherein the mesenchymal stem cells obtained in Step (e) are mesenchymal stem cells of CD34(-), CD45(-), HLA-DR(-), TRA-1-60(-), and TRA-1-81(-).