Claims

[Claim 1]

A method for producing neural crest cells and maintaining their multipotency, comprising the steps of:
(1) obtaining neural crest cells by inducing the differentiation of stem cells into the neural crest cells; and
(2) suspension-culturing the neural crest cells in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 3.0 μM to 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 2]

The production method according to claim 1, wherein the medium further comprises a TGFβ inhibitor.

[Claim 3]

The production method according to claim 1, wherein the medium is Chemically Defined Medium (CDM).

[Claim 4]

The production method according to claim 1, wherein the medium further comprises an epidermal growth factor.

[Claim 5]

The production method according to claim 1, wherein the GSK3β inhibitor is at least one member selected from the group consisting of CHIR99021, CP21R7, CHIR98014, LY2090314, kenpaullone, AR-AO144-18, TDZD-8, SB216763, BIO, TWS-119 and SB415286.

[Claim 6]

The production method according to claim 5, wherein the GSK3β inhibitor is CHIR99021.

[Claim 7]

The production method according to claim 2, wherein the TGFβ inhibitor is at least one member selected from the group

consisting of SB431542, A83-01, LDN193189, Wnt3a/BIO, BMP4, GW788388, SM16, IN-1130, GW6604 and SB505124.

[Claim 8]

The production method according to claim 1, wherein in the step (2), the neural crest cells are passaged every 5 to 8 days after inoculation.

[Claim 9]

A method for proliferating neural crest cells and maintaining their multipotency, comprising the step of:
(I) suspension-culturing the neural crest cells for a period of 35 days or longer in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 3.0 μM to 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 10]

A method for producing nerve cells, glial cells, mesenchymal stromal cells, bone cells, chondrocytes, corneal cells or pigment cells, comprising the steps of:
(i) suspension-culturing neural crest cells in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 3.0 μM or higher and lower than 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population; and(ii) differentiating the neural crest cells obtained in the step (i) into cells of at least one lineage selected from the group consisting of nerve cells, glial cells, mesenchymal stromal cells, bone cells, chondrocytes, corneal cells and pigment cells.

[Claim 11]

A method for culturing neural crest cells and extending their multipotency for a period of 63 days or longer, comprising the steps of:

(1) obtaining neural crest cell by inducing the differentiation of stem cells into the neural crest cells; and

(2) suspension-culturing the neural crest cells in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 3.0 μM to 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 12]

The production method according to claim 1, wherein the cultured neural crest cells that maintain multipotency over 77 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 80% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 13]

The method according to claim 9, wherein the cultured neural crest cells that maintain multipotency over 77 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 80% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 14]

The method according to claim 11, wherein the cultured neural crest cells that maintain multipotency over 77 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 80% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 15]

The production method according to claim 1, wherein the cultured neural crest cells that maintain multipotency over 121 days or longer, and wherein the cultured neural crest cells

comprise a ratio of at least 90% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 16]

The method according to claim 9, wherein the cultured neural crest cells that maintain multipotency over 121 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 90% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 17]

The method according to claim 11, wherein the cultured neural crest cells that maintain multipotency over 121 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 90% or more of neural crest cells that maintain multipotency to cultured cell population.