According to a fifth aspect, there is provided a cell produced by the method of any one of claims as described herein.

According to a sixth aspect, there is provided a method of culturing a cell comprising the step of contacting said cell with a cell culture medium comprising at least one of:

a) FGF

b) VEGF, or

c) HGF,

wherein the FGF, VEGF and HGF are each provided at a concentration of about 0.02ng/ml to 250ng/ml.

According to a seventh aspect, there is provided a cell culture medium for culturing a cell comprising at least one of FGF, VEGF, or HGF, wherein the FGF, VEGF and HGF are each provided at a concentration of about 0.02ng/ml to 250ng/ml.

According to an eighth aspect, there is provided a kit for use in the method as disclosed herein, comprising one or more containers of cell culture medium as claimed as disclosed herein, together with instructions for use.

According to an ninth aspect, there is provided the use of a cell as disclosed herein, for bio-therapeutic production.

According to an tenth aspect, there is provided the use of a cell as disclosed herein, for antibody production.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "ingredient" refers to any compound, whether of chemical or biological origin, that can be used in cell culture media to maintain the cell viability or promote the proliferation of cells. The terms "component," "nutrient" and ingredient" can be used interchangeably and are all meant to refer to such compounds. Typical ingredients that are used in cell culture media include amino acids, salts, metals, sugars, lipids, nucleic acids, hormones, vitamins, fatty acids,

proteins, hydrolysates, serum and the like. Other ingredients that promote or maintain cultivation of cells ex vivo can be selected by those of skill in the art, in accordance with the particular need.

The term "cytokine" refers to a compound that induces a physiological response in a cell, such as growth, differentiation, senescence, apoptosis, cytotoxicity or antibody secretion. Included in this definition of "cytokine" are growth factors, interleukins, colony-stimulating factors, interferons and lymphokines.

By "cell culture" or "culture" is meant the maintenance of cells in an artificial, in vitro environment. However, it is to be understood that the term "cell culture" is a generic term and may be used to encompass the cultivation not only of individual cells, but also of tissues, organs, organ systems or whole organisms, for which the terms "tissue culture," "organ culture," "organ system culture" or "organotypic culture" may occasionally be used interchangeably with the term "cell culture".

Claims

1. A method of culturing a cell comprising the step of contacting said cell with a cell culture medium comprising at least one of:

a) FGF

b) VEGF, or

c) HGF,

wherein the FGF, VEGF and HGF are each provided at a concentration of about 0.02ng/ml to 250ng/ml.

2. The method as claimed in claim 1, wherein the cell is a mammalian cell.

3. The method as claimed in claim 1, wherein the mammalian cell is an epithelial cell.

4. The method as claimed in claim 1 or claim 2, wherein the cell is selected from a CHO, MDCK, HEK, HeLa, A549, COS, Hep-G2, hybridoma, NSO, BHK, Vero and PerC6 or MCF-7 cell.

5. The method as claimed in claim 4, wherein the cell is a CHO cell.

6. The method as claimed in claim 5, wherein the CHO cell is a CHO-K1 cell or CHO-DG44 cell.

7. The method as claimed in claim ~~5 or~~ 6, wherein the CHO-K1 or CHO-DG44 expresses a recombinant protein.

8. The method as claimed in claim 6 or 7, wherein the CHO-K1 cell or CHO-DG44 is a single cell clone.

9. The method as claimed in any one of the preceding claims, wherein the culture medium comprises FGF, VEGF, and HGF.

10. The method as claimed in claim 9, wherein the culture medium comprises FGF-8, VEGF-c, and HGF.

~~11. The method of claim 10, wherein the FGF-8, VEGF-c and HGF are each provided at a concentration of about 0.02ng/ml to 250ng/ml.~~

~~12~~11. The method of claim ~~11~~10, wherein the FGF-8, VEGF-c and HGF are each provided at a concentration of about 10ng/ml to 100ng/ml.

~~13~~12. The method of claim ~~11~~ 10 or ~~12~~11, wherein the FGF-8 is at a concentration of about 100ng/ml.

~~14~~13. The method of claim ~~11~~ 10 or ~~12~~11, wherein the VEGF-c is at a concentration of about 100ng/ml.

~~15~~14. The method of claim ~~11~~ 10 or ~~12~~11, wherein the HGF is at a concentration of about 10ng/ml.

~~16~~15. The method of any one of claims 1 to ~~15~~14, wherein the culture medium is a serum-free medium.

~~17~~16. The method of claim ~~16~~15, wherein the culture medium is a serum-free medium supplemented with IGF, Insulin, EGF and HSA.

~~18~~17. The method as claimed in any one of claims 1 to ~~17~~16, wherein the cell is cultured for ~~about~~ 14 to ~~about~~ 28

days at ~~about~~ 30°C to ~~about~~ 37°C in ~~about~~ 0% to ~~about~~ 8% CO_2.

~~19~~18. The method as claimed in any one of claims 1 to ~~18~~17, wherein said method results in an increase in the cloning efficiency of the cultured cell by about 10% to about 26% relative to a cloned cell cultured in a cell culture medium that is not supplemented with at least one of FGF, VEGF, or HGF.

~~20~~19. A cell culture medium for culturing a cell comprising at least one of FGF, VEGF, or HGF, wherein the FGF, VEGF and HGF are each provided at a concentration of about 0.02ng/ml to 250ng/ml.

~~21~~20. The cell culture medium as claimed in claim ~~20~~19, wherein the medium comprises:

a. FGF-8,

b. VEGF-c, and

c. HGF.

~~22. The cell culture medium of claim 21, wherein the FGF-8, VEGF-c and HGF are each provided at a concentration of about 0.02ng/ml to 250ng/ml.~~

~~23~~21. The cell culture medium of claim ~~22~~20, wherein the FGF-8, VEGF-c and HGF are each provided at a concentration of about 10ng/ml to 100ng/ml.

~~24~~22. The cell culture medium of claim ~~22~~ 20 or ~~23~~21, wherein the FGF-8 is at a concentration of about 100ng/ml.

~~25~~23. The cell culture medium of claim ~~22~~ 20 or ~~23~~21, wherein the VEGF-c is at a concentration of about 100ng/ml.

~~26~~24. The cell culture medium of claim ~~22~~ 20 or ~~23~~21, wherein the HGF is at a concentration of about 10ng/ml.

~~27~~25. The cell culture medium of claims ~~20~~ 19 to ~~26~~ 24 wherein the cell culture medium is a serum free medium.

~~28~~26. The cell culture medium of claim ~~27~~25, wherein the cell culture medium is a serum free basal medium.

~~29~~27. A kit ~~when used~~for use in the method of any one of claims 1 to ~~19~~18, comprising one or more containers of cell culture medium as claimed in any one of claims ~~20~~ 19 to ~~28~~26, together with instructions for use.

~~30~~28. Use of a cell culture medium as claimed in any one of claims ~~20~~ 19 to ~~28~~ 26 for culturing a cell.

~~31~~29. A cell produced by the method of any one of claims 1 to ~~19~~18.

~~32~~30. Use of a cell according to claim ~~31~~29, for bio-therapeutic production.

~~33~~31. Use of a cell according to claim ~~31~~29, for antibody production.