Claims

1. A culture medium for growing anchorage-dependent cells comprising:

the amino acids L-Arginine; L-Cystine; L-Cysteine; L-Glutamine; Glycine; L-Histidine; L-Isoleucine; L-Leucine; L-Lysine; L-Methionine; L-Phenylalanine; L-serine; L-Threonine; L-tryptophan; L-tyrosine; L-Valine; L-Alanine; L-Asparagine; L-Aspartic Acid; L-Glutamic Acid; L-Proline; and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; i-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, and one or more sodium phosphate salts;

the nucleosides thymidine, adenosine, cytidine, uridine and guanosine;

insulin; transferrin; lipoic acid/thioctic acid; ethanolamine; sodium selenite; and one or more energy sources.

2. The culture medium of claim 1, wherein the culture medium comprises:

at least about 0.05 g/L of L-Arginine; at least about 0.02 g/L of L-Cystine; at least about 0.2 g/L of L-Glutamine; at least about 0.01 g/L Glycine; at least about 0.02 g/L of L-Histidine; at least about 0.09 g/L of L-Isoleucine; at least about 0.09 g/L of L-Leucine; at least about 0.09 g/L of L-Lysine; at least about 0.02 g/L of L-Methionine; at least about 0.05 g/L of L-Phenylalanine; at least about 0.03 g/L of L-serine; at least about 0.08 g/L of L-Threonine; at least about 0.009 g/L of L-tryptophan; at least about 0.08 g/L of L-tyrosine; at least about 0.08 g/L of L-Valine; at least about 0.005 g/L L-Cysteine; at least about 0.0004 g/L of L-Alanine; at least about 0.01 g/L of L-Asparagine; at least about 0.006 g/L of L-Aspartic Acid; at least 0.03 g/L of L-Glutamic Acid; at least about 0.005 g/L L-Proline; and at least about 0.0003 g/L of L-Taurine;

from about 5×10^{-6} g/L to about 0.015 g/L of each of the vitamins;

at least about 0.05 g/L of Calcium Chloride, Anhydrous, at least about 0.1 g/L of potassium chloride; at least about 0.2 g/L of magnesium sulfate, at least about 0.08 g/L of sodium phosphate, monobasic, H_2O and at least about 0.0005 g/L of sodium phosphate, dibasic heptahydrate ($Na_2HPO_4.7H_2O$);

at least about 0.0001 g/L of thymidine and least about 0.005 g/L of each of adenosine, cytidine, uridine, and guanosine; and

at least 0.003 g/L of insulin, at least 0.05 g/L of transferrin, at least about 5 x 10^{-6} g/L of lipoic acid/thioctic acid, at least 0.05 g/L of ethanolamine and at least about 0.00004 g/L of sodium selenite.

3. A serum-free nutrient solution for growing anchorage dependent cells comprising:

the amino acids L-Arginine, L-Cystine, L-Cysteine, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-serine, L-Threonine, L-tryptophan, L-tyrosine, L-Valine, L-Alanine, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, L-Proline and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts sodium phosphate, monobasic and sodium phosphate, dibasic heptahydrate;

the nucleosides adenosine, cytidine, uridine and guanosine; and

insulin; transferrin; lipoic acid/thioctic acid; ethanolamine; and sodium selenite.

4. A method of culturing isolated umbilical cord tissue-derived cells comprising:

a. growing umbilical cord tissue-derived cells seeded on microcarriers in a culture medium comprising amino acids, vitamins, salts, nucleosides, lipoic/thioctic acid, ethanolamine, insulin, transferrin, sodium selenium, wherein the culture medium is supplemented with serum, for a sufficient period of time to allow for the cells to achieve a desired initial population density;

b. adding a serum-free nutrient solution after the cells have achieved the desired initial population density, wherein the serum-free nutrient solution comprises amino acids, vitamins, salts, insulin, transferrin, ethanolamine, lipoic acid/thioctic acid, sodium selenium; and

c. growing the cells for a sufficient period of time to allow for the cells to achieve a desired final population density;

wherein the umbilical cord tissue-derived cells are isolated from human umbilical cord tissue substantially free of blood, are capable of self-renewal and expansion in culture, have the potential to differentiate, express CD13, CD90, HLA-ABC, and do not express CD34, CD117 and HLA-DR.

5. The method of claim 4 wherein the method further comprises seeding the cells on the microcarriers.

6. The method of claim 4, wherein the method further comprises isolating the cells after step c.

7. The method of claim 4, wherein the method does not require medium exchange.

8. The method of claim 4, wherein the method is carried out in a spinner flask culture system.

9. The method of claim 4, wherein the characteristics of the cells before and after culturing are substantially the same.

10. The method of claim 9, wherein the characteristics of the cells before and after culturing are the same.

11. The method of claim 4, wherein the desired initial population density is achieved after 3 to 4 days.

12. The method of claim 4, wherein the microcarriers have an amine treated surface.

13. The method of claim 4, wherein the culture medium comprises:

the amino acids L-Arginine; L-Cystine; L-Cysteine; L-Glutamine; Glycine; L-Histidine; L-Isoleucine; L-Leucine; L-Lysine; L-Methionine; L-Phenylalanine; L-serine; L-Threonine; L-tryptophan; L-tyrosine; L-Valine; L-Alanine; L-Asparagine; L-Aspartic Acid; L-Glutamic Acid; L-Proline; and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, and one or more sodium phosphate salts;

the nucleosides thymidine, adenosine, cytidine, uridine and guanosine; and

insulin; transferrin; lipoic acid/thioctic acid; ethanolamine; sodium selenite; and one or more energy sources.

14. The method of claim 13, wherein the one or more energy sources are D-glucose and sodium pyruvate.

15. The method of claim 13, wherein the culture medium comprises:

at least about 0.05 g/L of L-Arginine; at least about 0.02 g/L of L-Cystine; at least about 0.2 g/L of L-Glutamine; at least about 0.01 g/L Glycine; at least about 0.02 g/L of L-Histidine; at least about 0.09 g/L of L-Isoleucine; at least about 0.09 g/L of L-Leucine; at least about 0.09 g/L of L-Lysine; at least about 0.02 g/L of L-Methionine; at least about 0.05 g/L of L-Phenylalanine; at least about 0.03 g/L of L-serine; at least about 0.08 g/L of L-Threonine; at least about 0.009 g/L of L-tryptophan; at least about 0.08 g/L of L-tyrosine; at least about 0.08 g/L of L-Valine; at least about 0.005 g/L L-Cysteine; at least about 0.0004 g/L of L-Alanine; at least about 0.01 g/L of Asparagine; at least about 0.006 g/L of L-Aspartic Acid; at least 0.03 g/L of L-Glutamic Acid; at least about 0.005 g/L L-Proline; and at least about 0.0003 g/L of L-Taurine;

from about 5×10^{-6} g/L to about 0.015 g/L of each of the vitamins;

at least about 0.05 g/L of Calcium Chloride, Anhydrous, at least about 0.1 g/L of potassium chloride; at least about 0.2 g/L of magnesium sulfate, at least about 0.08 g/L of sodium phosphate, monobasic, H_2O and at least about 0.0005 g/L of sodium phosphate, dibasic heptahydrate ($Na_2HPO_4 \cdot H_2O$);

at least about 0.0001 g/L of thymidine and least about 0.005 g/L of each of adenosine, cytidine, uridine, and guanosine; and

at least 0.003 g/L of insulin, at least 0.05 g/L of transferrin, at least about 5×10^{-6} g/L of lipoic acid/thioctic acid, at least 0.05 g/L of ethanolamine and at least about 0.00004 g/L of sodium selenite.

16. The method of claim 13, wherein the culture medium is supplemented with 2 to 20% of FBS.

17. The method of claim 13, wherein the culture medium is supplemented with about 7.5%, about 10%, or about 15% of FBS.

18. The method of claim 13, wherein the culture medium further comprises putrescine, a stabilizer, and/or a foaming agent.

19. The method of claim 4, wherein in the serum-free nutrient solution comprises:

the amino acids L-Arginine, L-Cystine, L-Cysteine, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-serine, L-Threonine, L-tryptophan, L-tyrosine, L-Valine, L-Alanine, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, L-Proline and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts sodium phosphate, monobasic and sodium phosphate, dibasic heptahydrate;

the trace minerals Copper(II)sulfate pentahydrate ($CuSO_4.5H_2O$), Zinc sulfate, heptahydrate, ($ZnSO_4.7H_2O$);

the nucleosides adenosine, cytidine, uridine and guanosine; and

insulin; transferrin; ethanolamine; lipoic acid/thioctic acid; and sodium selenite.

20. The method of claim 19, wherein the serum-free solution further comprises putrescine, a stabilizer, and/or a foaming agent.

Claims

1. A culture medium for growing anchorage-dependent cells comprising:

the amino acids L-Arginine; L-Cystine; L-Cysteine; L-Glutamine; Glycine; L-Histidine; L-Isoleucine; L-Leucine; L-Lysine; L-Methionine; L-Phenylalanine; L-serine; L-Threonine; L-tryptophan; L-tyrosine; L-Valine; L-Alanine; L-Asparagine; L-Aspartic Acid; L-Glutamic Acid; L-Proline; and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, and one or more sodium phosphate salts;

the nucleosides thymidine, adenosine, cytidine, uridine and guanosine;

insulin; transferrin; lipoic acid/thioctic acid; ethanolamine; sodium selenite; and one or more energy sources.

2. The culture medium of claim 1, wherein the culture medium comprises:

at least about 0.05 g/L of L-Arginine; at least about 0.02 g/L of L-Cystine; at least about 0.2 g/L of L-Glutamine; at least about 0.01 g/L Glycine; at least about 0.02 g/L of L-Histidine; at least about 0.09 g/L of L-Isoleucine; at least about 0.09 g/L of L-Leucine; at least about 0.09 g/L of L-Lysine; at least about 0.02 g/L of L-Methionine; at least about 0.05 g/L of L-Phenylalanine; at least about 0.03 g/L of L-serine; at least about 0.08 g/L of L-Threonine; at least about 0.009 g/L of L-tryptophan; at least about 0.08 g/L of L-tyrosine; at least about 0.08 g/L of L-Valine; at least about 0.005 g/L L-Cysteine; at least about 0.0004 g/L of L-Alanine; at least about 0.01 g/L of L-Asparagine; at least about 0.006 g/L of L-Aspartic Acid; at least 0.03 g/L of L-Glutamic Acid; at least about 0.005 g/L L-Proline; and at least about 0.0003 g/L of L-Taurine;

from about 5×10^{-6} g/L to about 0.015 g/L of each of the vitamins;

at least about 0.05 g/L of Calcium Chloride, Anhydrous, at least about 0.1 g/L of potassium chloride; at least about 0.2 g/L of magnesium sulfate, at least about 0.08 g/L of sodium phosphate, monobasic, H_2O and at least about 0.0005 g/L of sodium phosphate, dibasic heptahydrate ($Na_2HPO_4.7H_2O$);

at least about 0.0001 g/L of thymidine and least about 0.005 g/L of each of adenosine, cytidine, uridine, and guanosine; and

at least 0.003 g/L of insulin, at least 0.05 g/L of transferrin, at least about 5 x 10^{-6} g/L of lipoic acid/thioctic acid, at least 0.05 g/L of ethanolamine and at least about 0.00004 g/L of sodium selenite.

3. A serum-free nutrient solution for growing anchorage dependent cells comprising:

the amino acids L-Arginine, L-Cystine, L-Cysteine, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-serine, L-Threonine, L-tryptophan, L-tyrosine, L-Valine, L-Alanine, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, L-Proline and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts sodium phosphate, monobasic and sodium phosphate, dibasic heptahydrate;

the nucleosides adenosine, cytidine, uridine and guanosine; and

insulin; transferrin; lipoic acid/thioctic acid; ethanolamine; and sodium selenite.

4. A method of culturing isolated umbilical cord tissue-derived cells comprising:

a. growing umbilical cord tissue-derived cells seeded on microcarriers in a culture medium comprising amino acids, vitamins, salts, nucleosides, lipoic/thioctic acid, ethanolamine, insulin, transferrin, sodium selenium, wherein the culture medium is supplemented with serum, for a sufficient period of time to allow for the cells to achieve a desired initial population density;

b. adding a serum-free nutrient solution after the cells have achieved the desired initial population density, wherein the serum-free nutrient solution comprises amino acids, vitamins, salts, insulin, transferrin, ethanolamine, lipoic acid/thioctic acid, sodium selenium; and

c. growing the cells for a sufficient period of time to allow for the cells to achieve a desired final population density;

wherein the umbilical cord tissue-derived cells are isolated from human umbilical cord tissue substantially free of blood, are capable of self-renewal and expansion in culture, have the potential to differentiate, express CD13, CD90, HLA-ABC, and do not express CD34, CD117 and HLA-DR.

5. The method of claim 4 wherein the method further comprises seeding the cells on the microcarriers.

6. The method of claim 4, wherein the method further comprises isolating the cells after step c.

7. The method of claim 4, wherein the method does not require medium exchange.

8. The method of claim 4, wherein the method is carried out in a spinner flask culture system.

9. The method of claim 4, wherein the characteristics of the cells before and after culturing are substantially the same.

10. The method of claim 9, wherein the characteristics of the cells before and after culturing are the same.

11. The method of claim 4, wherein the desired initial population density is achieved after 3 to 4 days.

12. The method of claim 4, wherein the microcarriers have an amine treated surface.

13. The method of claim 4, wherein the culture medium comprises:

the amino acids L-Arginine; L-Cystine; L-Cysteine; L-Glutamine; Glycine; L-Histidine; L-Isoleucine; L-Leucine; L-Lysine; L-Methionine; L-Phenylalanine; L-serine; L-Threonine; L-tryptophan; L-tyrosine; L-Valine; L-Alanine; L-Asparagine; L-Aspartic Acid; L-Glutamic Acid; L-Proline; and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, and one or more sodium phosphate salts;

the nucleosides thymidine, adenosine, cytidine, uridine and guanosine; and

insulin; transferrin; lipoic acid/thioctic acid; ethanolamine; sodium selenite; and one or more energy sources.

14. The method of claim 13, wherein the one or more energy sources are D-glucose and sodium pyruvate.

15. The method of claim 13, wherein the culture medium comprises:

at least about 0.05 g/L of L-Arginine; at least about 0.02 g/L of L-Cystine; at least about 0.2 g/L of L-Glutamine; at least about 0.01 g/L Glycine; at least about 0.02 g/L of L-Histidine; at least about 0.09 g/L of L-Isoleucine; at least about 0.09 g/L of L-Leucine; at least about 0.09 g/L of L-Lysine; at least about 0.02 g/L of L-Methionine; at least about 0.05 g/L of L-Phenylalanine; at least about 0.03 g/L of L-serine; at least about 0.08 g/L of L-Threonine; at least about 0.009 g/L of L-tryptophan; at least about 0.08 g/L of L-tyrosine; at least about 0.08 g/L of L-Valine; at least about 0.005 g/L L-Cysteine; at least about 0.0004 g/L of L-Alanine; at least about 0.01 g/L of Asparagine; at least about 0.006 g/L of L-Aspartic Acid; at least 0.03 g/L of L-Glutamic Acid; at least about 0.005 g/L L-Proline; and at least about 0.0003 g/L of L-Taurine;

from about 5×10^{-6} g/L to about 0.015 g/L of each of the vitamins;

at least about 0.05 g/L of Calcium Chloride, Anhydrous, at least about 0.1 g/L of potassium chloride; at least about 0.2 g/L of magnesium sulfate, at least about 0.08 g/L of sodium phosphate, monobasic, H_2O and at least about 0.0005 g/L of sodium phosphate, dibasic heptahydrate ($Na_2HPO_4.7H_2O$);

at least about 0.0001 g/L of thymidine and least about 0.005 g/L of each of adenosine, cytidine, uridine, and guanosine; and

at least 0.003 g/L of insulin, at least 0.05 g/L of transferrin, at least about 5×10^{-6} g/L of lipoic acid/thioctic acid, at least 0.05 g/L of ethanolamine and at least about 0.00004 g/L of sodium selenite.

16. The method of claim 15, wherein the culture medium is supplemented with 2 to 20% of FBS.

17. The method of claim 15, wherein the culture medium is supplemented with about 7.5%, about 10%, or about 15% of FBS.

18. The method of claim 15, wherein the culture medium further comprises putrescine, a stabilizer, and/or a foaming agent.

19. The method of claim 6, wherein in the serum-free nutrient solution comprises:

the amino acids L-Arginine, L-Cystine, L-Cysteine, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-serine, L-Threonine, L-tryptophan, L-tyrosine, L-Valine, L-Alanine, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, L-Proline and L-Taurine;

the vitamins D-calcium pantothenate; choline chloride; folic acid; I-inositol; niacinamide; pyridoxal; riboflavin; thiamine; d-Biotin; pyridoxine; and Vitamin B_{12} (cyanocobalamin);

the salts sodium phosphate, monobasic and sodium phosphate, dibasic heptahydrate;

the trace minerals Copper(II)sulfate pentahydrate ($CuSO_4.5H_2O$), Zinc sulfate, heptahydrate, ($ZnSO_4.7H_2O$);

the nucleosides adenosine, cytidine, uridine and guanosine; and

insulin; transferrin; ethanolamine; lipoic acid/thioctic acid; and sodium selenite.

20. The method of claim 21, wherein the serum-free solution further comprises putrescine, a stabilizer, and/or a foaming agent.

21. The serum-free nutrient solution of claim 3, wherein the serum-free nutrient solution lacks an energy substrate.

22. The method of claim 4, wherein the serum-free nutrient solution lacks an energy substrate.