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(71) Applicant: **TRIZELL LTD.** [GB/GB]; Sanderum House Oakley Road, Chinnor, Oxfordshire OX39 4TW (GB).

(71) Applicant *(for US only)*: **PHARMACEUTICAL PATENT ATTORNEYS, LLC** [US/US]; 55 Madison Avenue, 4th Floor, Morristown, NJ 07960 (US).

(72) Inventors: **LESCH, Hanna, P.**; Microkatu 1S, 70210 Kuopio (FI). **VALONEN, Piia, Kristiina**; Maaherrankatu 1 B 38, 70100 Kuopio (FI). **RÄSÄNEN, Eva, Kristiina**; Heinäahontie 9, 70870 Hiltulanlahti (FI). **TUUNANEN, Tarja, Hannele**; Maitotie 7 C 19, 70780 Kuopio (FI). **KARNAATTU, Milla Jonna, Karoliina**; Lehväkuja 6, 70150 Kuopio (FI). **MÜLLER, Achim**; Susiniementie 238, 71150 Vartiala (FI). **KARHINEN, Minna, Kristiina**; Sammonkatu 28 A16, 70500 Kuopio (FI).

(74) Agent: **POHL, Mark**; Pharmaceutical Patent Attorneys, LLC, 55 Madison Avenue, 4th Floor, Morristown, NJ 07960-7397 (US).

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(54) Title: FIXED-BED BIOREACTOR WITH CONSTANT-FLOW PUMP/ TUBING SYSTEM

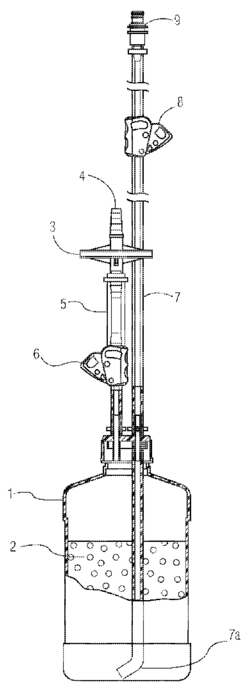

FIG. 2

(57) Abstract: We have modified a commercially-available adherent cell culture bioreactor in several ways to increase productivity of cultured cells, while decreasing contamination risk. We found that modifying a commercially-available adherent cell culture bioreactor to provide for slower cell culture medium flow unexpectedly and dramatically increases the productivity of the cultured adherent cells. We also developed a new sampling manifold configuration and new way of taking samples, to reduce contamination risk.

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[Continued on next page]

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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AMENDED CLAIMS
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We claim:

1. An adherent cell culture bioreactor comprising a substrate for adherent cell culture and a pump able to provide a constant-rate output of cell culture media of less than about 50 mL of media per 1,800 cells per day.

2. The adherent cell culture bioreactor of claim 1, the pump able to provide a minimum constant-rate output of not more than about 16.7 mL of media per 1,800 cells per day.

3. The adherent cell culture bioreactor of claim 1, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

4. The adherent cell culture bioreactor of claim 3, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

5. The adherent cell culture bioreactor of claim 1, configured to contain at least about 20 liters of cell culture media.

6. A recombinant polypeptide produced by culturing adherent producer cells in the adherent cell bioreactor of claim 1.

7. The recombinant polypeptide of claim 6, where the polypeptide comprises virus.

8. The recombinant polypeptide of claim 7, where the virus comprises a viral capsid containing a non-viral transgene.

9. A method for culturing adherent cells on a substrate comprising:

a. obtaining the bioreactor of claim 1, and then

b. adding to the bioreactor cell culture media and cells, and then

c. culturing the cells in adherent mode on the substrate, while circulating the cell culture media about the cells at a rate of less than about 50 mL of media per 1,800 cells per day.

10. The method of claim 9, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

11. The method of claim 9, the bioreactor configured to contain at least about 20 liters of cell culture media.

12. An adherent-cell bioreactor comprising a container containing a substrate for adherent cell culture and a pump configured to be able to provide a constant-rate output of cell culture medium at a pump output volume sufficient to maintain at a relatively constant concentration in said cell culture medium a parameter selected from the group consisting of: concentration of lactate in said medium of not more than about 1.58 grams / liter; concentration of glucose in said medium of between about 0.5 and 1.0 grams / liter; and concentration of glucose in said medium of not less than about 2.89 grams / liter.

13. The adherent cell culture bioreactor of claim 12, configured to contain at least about 20 liters of cell culture media.

14. The adherent cell culture bioreactor of claim 12, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

15. The adherent cell culture bioreactor of claim 14, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

16. The adherent cell bioreactor of claim 12, the pump configured to be able to provide a constant-rate output of cell culture medium at a pump output volume sufficient to maintain in said cell culture medium a concentration of lactate in said medium of not more than about 1.58 grams / liter.

17. The adherent cell bioreactor of claim 12, the pump configured to be able to provide a constant-rate output of cell culture medium at a pump output volume sufficient to maintain in said cell culture medium a concentration of glucose in said medium of between about 0.5 and 1.0 grams / liter;

18. The adherent cell bioreactor of claim 12, the pump configured to be able to provide a constant-rate output of cell culture medium at a pump output volume sufficient to maintain in said cell culture medium a concentration of glucose in said medium of not less than about 2.89 grams / liter.

19. A recombinant polypeptide produced by culturing adherent producer cells in the adherent cell bioreactor of claim 12.

20. The recombinant polypeptide of claim 19, where the polypeptide comprises virus.

21. The recombinant polypeptide of claim 20, where the virus comprises a viral capsid containing a non-viral transgene.

22. A method for culturing adherent cells on a substrate comprising:

a. obtaining the bioreactor of claim 12, and then

b. adding to the bioreactor cell culture media and cells, and then

c. culturing the cells in adherent mode on the substrate, while circulating the cell culture media about the cells at a rate to maintain in said cell culture medium a parameter selected from the group consisting of: concentration of lactate in said medium of not more than about 1.58 grams / liter; concentration of glucose in said medium of between about 0.5 and 1.0 grams / liter; and concentration of glucose in said medium of not less than about 2.89 grams / liter.

23. The method of claim 22, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

24. The method of claim 23, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

25. The method of claim 22, the bioreactor configured to contain at least about 20 liters of cell culture media.

26. An adherent cell culture bioreactor providing a substrate for adherent cell growth and configured to contain cell culture media and having a pump able to pump said media, the pump configured to be able to provide a constant-rate output of media at an output volume of less than about 50 mL media per m^2 substrate area per day.

27. The adherent cell culture bioreactor of claim 26, configured to contain at least about 20 liters of cell culture media.

28. The adherent cell culture bioreactor of claim 26, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

29. The adherent cell culture bioreactor of claim 28, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

30. The adherent cell culture bioreactor of claim 26, wherein the pump is connected to the substrate via a tube having an interior diameter sized to accommodate the pump output volume of fluid without the formation of air bubbles inside the tube.

31. A recombinant polypeptide produced by culturing adherent producer cells in the adherent cell bioreactor of claim 26.

32. The recombinant polypeptide of claim 31, where the polypeptide comprises virus.

33. The recombinant polypeptide of claim 32, where the virus comprises a viral capsid containing a non-viral transgene.

34. A method for culturing adherent cells on a substrate comprising:

a. obtaining the bioreactor of claim 26, and then

b. adding to the bioreactor cell culture media and cells, and then

c. culturing the cells in adherent mode on the substrate, while circulating the cell culture media about the cells at a rate of less than about 50 mL of media per m^2 substrate area per day.

35. The method of claim 34, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

36. The method of claim 35, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

37. The method of claim 34, the bioreactor configured to contain at least about 20 liters of cell culture media.

38. A method comprising:

a. obtaining an adherent cell culture bioreactor having a substrate for adherent cell culture and a first sampling tube having an input end and an output end, the input end of the first sampling tube in communication with the substrate whereby liquid can flow from the substrate into the input end of the first sampling tube, through the first sampling tube and exit the bioreactor at the output end of the first sampling tube; and then

b. adding cell culture media to the bioreactor, seeding cells into the bioreactor and culturing cells adherent to the substrate, and

c. flowing the cell culture media through the first sampling tube, and then

d. removing from the first sampling tube output end at least one removed portion of cell culture media; and then

e. permanently sealing the first sampling tube without returning into the output end of the sampling tube a material amount of the removed portion of cell culture media.

39. The method of claim 38, the adherent cell culture bioreactor further comprising a second sampling tube having an input end and an output end, the input end in communication with the substrate whereby liquid can flow from the substrate into the input end of the second sampling tube, through the second sampling tube and exit the bioreactor at the output end of the sampling tube; the method further comprising:

f. flowing the cell culture media through the second sampling tube, and then

g. removing from the second sampling tube output end a second removed portion of cell culture media; and then

h. permanently sealing the second sampling tube without returning into the output end of the second sampling tube a material amount of the removed cell culture media.

40. The method of claim 38, wherein the bioreactor is configured to contain at least about 20 liters of cell culture media.

41. The method of claim 38, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

42. The method of claim 39, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

43. The method of claim 39, wherein (b) the cells adherent to the substrate express a recombinant polypeptide.

44. The recombinant polypeptide produced by the method of claim 43.

45. The recombinant polypeptide of claim 44, where the polypeptide comprises virus.

46. The recombinant polypeptide of claim 45, where the virus comprises a viral capsid containing a non-viral transgene.

47. An adherent cell culture bioreactor comprising:

a. a substrate for adherent cell culture in flowable communication with at least two pairs of closable sampling tubes, whereby cell culture media can flow from the substrate through each sampling tube, and whereby each sampling tube may be aseptically closed after cell culture media flows from the substrate through the sampling tube, to prevent further media flow through the sampling tube; and

b. at least one of each pair of sampling tubes configured to be aseptically attached to a detachable sampling container.

48. The bioreactor of claim 47, configured to contain at least about 20 liters of cell culture media.

49. The bioreactor of claim 50, further comprising additional pairs of closable sampling tubes, at least one of the additional pairs of sampling tubes configured to be aseptically attached to a detachable sampling container.

50. The bioreactor of claim 47, further comprising a third pair of closable sampling tubes, at least one of the third pair of sampling tubes configured to be aseptically attached to a detachable sampling container.

51. The bioreactor of claim 47, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

52. The bioreactor of claim 51, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

53. A recombinant polypeptide produced by the bioreactor of claim 47.

54. The recombinant polypeptide of claim 53, where the polypeptide comprises virus.

55. The recombinant polypeptide of claim 54, where the virus comprises a viral capsid containing a non-viral transgene.

56. An adherent cell culture bioreactor vessel having a substrate for adherent cell culture and a pump able to provide a constant-rate output of cell culture media sufficient to exchange the cell culture media in the vessel less than once every about 3 ½ days.

57. The adherent cell culture bioreactor of claim 56, the pump able to provide a constant-rate output of cell culture media sufficient to exchange the cell culture media in the vessel less than once every about 4 ½ days.

58. The adherent cell culture bioreactor of claim 56, the substrate providing at least about 60 m^2 of surface area for adherent cell culture.

59. The adherent cell culture bioreactor of claim 58, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

60. The adherent cell culture bioreactor of claim 56, configured to contain at least about 20 liters of cell culture media.

61. A recombinant polypeptide produced by culturing adherent producer cells in the adherent cell bioreactor of claim 56.

62. The recombinant polypeptide of claim 61, where the polypeptide comprises virus.

63. The recombinant polypeptide of claim 62, where the virus comprises a viral capsid containing a non-viral transgene.

64. A method for culturing adherent cells on a substrate comprising:

a. obtaining the bioreactor of claim 56, and then

b. adding to the bioreactor cell culture media and cells, and then

c. culturing the cells in adherent mode on the substrate, while circulating the cell culture media about the cells at a rate of less than about 50 mL of media per 1,800 cells per day.

65. The method of claim 64, the substrate providing at least about 400 m^2 of surface area for adherent cell culture.

66. The method of claim 64, the bioreactor configured to contain at least about 20 liters of cell culture media.

PHARMACEUTICAL PATENT ATTORNEYS, LLC
www.LicensingLaw.Net

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Morristown, NJ 07960-7397 USA

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19 November 2017

The World Intellectual Property Organization
International Bureau
34, chemin des Colombettes
1221 Geneva 20 SWITZERLAND
BY ePCT FILING

Re: **STATEMENT UNDER ARTICLE 19 / RULE 46**
PCT/US2017/025681, international filing date 03 April 2017

Sirs:

The 02 October *Written Opinion* correctly notes that ZHANG [0382] echoes the art in teaching to culture cells in a glucose concentration decreasing from 5.4 to 3.0 g/L over a week. In contrast, the inventors found it advantageous that glucose is "maintained at a relatively constant concentration." *See Specification* lines 146-47. ZHANG [0382] fails to anticipate Claim 12 because ZHANG fails to teach maintaining "a relatively constant concentration." ZHANG fails to render obvious because ZHANG fails to recognize that maintaining "a relatively constant concentration" is results-critical.

The 02 October *Written Opinion* also correctly notes that ZHANG [0013] teaches to maintain glucose concentration "between about 0.7 and about 1.7 g/L." The Examiner correctly notes the range taught by ZHANG partially overlaps the range of claim 12. ZHANG, however, fails to recognize the claimed low range is results critical. ZHANG thus fails to anticipate, *see* M.P.E.P. § 2131.03(II), but evinces *prima facie* obviousness, *see* M.P.E.P. § 2144.05(I). Applicant respectfully believes *prima facie* obviousness is rebutted by Applicant's experimental data showing, for example, a 49.4% increase in productivity, *see Specification* line 133.

The 02 October *Written Opinion* correctly notes that STARBARD Figure 4 elements 10, 14, 15 and 16 is similar to the instant sampling apparatus. In contrast to STARBARD, however, the instant *Specification* teaches the necessity of flushing the line before taking a sample. *See* lines 273-93. STARBARD fails to recognize the need to flush. STARBARD thus fails to disclose more than one pair of sampling tubes. Applicant here amends the claims accordingly. STARBARD fails to anticipate because STARBARD Figure 4 element 16 shows only one pair. STARBARD fails to render obvious because STARBARD fails to recognize the need to flush, thus provides no motive to make the claimed modification.

The 02 October *Written Opinion* correctly notes that GENZYME fails to teach the claimed slow perfusion rate. GENZYME fails to recognize flow rate as a results-critical variable. Indeed, GENZYME expressly *discourages* slow flow. While instant Claim 56 requires flow so slow it takes about 3½ days to completely exchange medium, GENZYME [0223] says to take only 1 day, and as the cells grow *increase* the rate.

Respectfully submitted,

PHARMACEUTICAL PATENT ATTORNEYS, LLC

/s/
Mark POHL, USPTO Reg. No. 35,325

cc w/ enclosures:
ISA/US, Commissioner for Patents, Alexandria, VA 22313-1450 USA