combined with any of the above embodiments, the density of oxygen (DO) in the first cell culture medium during the steps of culturing an adherent cell in a fixed bed containing a macrocarrier, where the cell is cultured in a first cell culture medium and/or inoculating the cell with the Enterovirus A virus under conditions in which the Enterovirus A virus infects the cell is maintained above about 50%. In some embodiments that may be combined with any of the above embodiments, the density of oxygen (DO) in the second cell culture medium during the step of culturing the infected cell in the fixed bed in a second cell culture medium under conditions in which the infected cell produces the Enterovirus A virus is maintained above about 50%. In some embodiments that may be combined with any of the above embodiments, the fixed bed has a bed height of about 2 cm. In some embodiments that may be combined with any of the above embodiments, the fixed bed has a bed height of about 10 cm. In some embodiments that may be combined with any of the above embodiments, the macrocarrier is a microfiber matrix. In some embodiments that may be combined with any of the above embodiments, the macrocarrier has a porosity between about 60% and 99%. In some embodiments that may be combined with any of the above embodiments, the porosity is between about 80% and about 90%. In some embodiments that may be combined with any of the above embodiments, the macrocarrier has a surface area accessible to the cell of between about 150 cm^2/cm^3 and about 1000 cm^2/cm^3. In some embodiments that may be combined with any of the above embodiments, at least 5.0×10^7 TCID50/mL of the Enterovirus A virus is harvested in the step of harvesting the Enterovirus A virus produced by the cell. In some embodiments that may be combined with any of the above embodiments, the methods further include inactivating the Enterovirus A with one or more of beta-propiolactone (BPL), formalin, or binary ethylenimine (BEI). In some embodiments that may be combined with any of the above embodiments, the Enterovirus A virus is an attenuated virus.

[0013] Other embodiments of the present disclosure relate to an Enterovirus A virus produced by the method of any of the above embodiments. In some embodiments that may be combined with any of the above embodiments, the virus contains one or more antigens. In some embodiments that may be combined with any of the above embodiments, the virus has been inactivated with one or more of beta-propiolactone (BPL), formalin, or binary ethylenimine (BEI).

[0013a] Other embodiments of the present disclosure relate to a composition comprising the virus disclosed herein. Other embodiments of the present disclosure relate to an immunogenic composition comprising the virus disclosed herein. Other embodiments of the present disclosure relate to a vaccine comprising the virus disclosed herein.

[0014] Other embodiments of the present disclosure relate to a composition containing an Enterovirus A virus produced by the method of any of the above embodiments. Other embodiments

CLAIMS

What is claimed is:

1. A method for producing an Enterovirus A virus, comprising:

(a) culturing an adherent cell in a fixed bed comprising a macrocarrier, wherein the cell is cultured in a first cell culture medium;

(b) inoculating the cell with the Enterovirus A virus under conditions in which the Enterovirus A virus infects the cell;

(c) culturing the infected cell in the fixed bed in a second cell culture medium under conditions in which the infected cell produces the Enterovirus A virus; and

(d) harvesting the Enterovirus A virus produced by the cell.

2. The method of claim 1, wherein the cell is a Vero cell.

3. The method of any one of claims 1-2, wherein the Enterovirus A virus is selected from the group consisting of EV71, CA6, and CA16.

4. The method of claim 3, wherein the Enterovirus A virus comprises at least one non-human cell adaptation mutation.

5. The method of claim 4, wherein:

(a) the Enterovirus A virus is EV71, and wherein the at least one non-human cell adaptation mutation comprises a VP1 polypeptide mutation; or

(b) the Enterovirus A virus is CA6, and wherein the at least one non-human cell adaptation mutation comprises a mutation selected from the group consisting of a VP1 polypeptide mutation, a VP2 polypeptide mutation, a VP3 polypeptide mutation, and a 5' untranslated region (UTR) mutation; or

(c) the Enterovirus A virus is CA16, and wherein the at least one non-human cell adaptation mutation comprises a mutation selected from the group consisting of a 2A polypeptide mutation, a VP1 polypeptide mutation, a VP2 polypeptide mutation, and a 5' untranslated region (UTR) mutation.

6. The method of any one of claims 1-5, wherein between 4,000 cells/cm^2 and 16,000 cells/cm^2 are cultured in step (a).

7. The method of any one of claims 1-5, wherein between 100,000 cells/cm^2 and 800,000 cells/cm^2 are inoculated in step (b).

8. The method of any one of claims 1-7, wherein the first cell culture medium comprises serum.

9. The method of claim 8, wherein the first cell culture medium comprises less than 10% fetal bovine serum and the cell is not adapted to serum free medium.

10. The method of any one of claims 1-9, wherein the first cell culture medium and the second cell culture medium are different.

11. The method of claim 10, further comprising, between steps (a) and (b), removing the first cell culture medium and rinsing the cell with the second culture medium.

12. The method of any one of claims 1-11, wherein the second cell culture medium is a serum-free medium.

13. The method of any one of claims 1-12, wherein the cell is inoculated with the Enterovirus A virus at a multiplicity of infection (MOI) of between 0.025 and 0.0009.

14. The method of any one of claims 1-13, wherein the cell is cultured during steps (a), (b), and/or (c) at a volume/surface ratio of 0.3 mL/cm^2.

15. The method of any one of claims 1-14, wherein the lactate concentration in the first cell culture medium during steps (a) and/or (b) does not exceed 25 mM.

16. The method of any one of claims 1-15, wherein the lactate concentration in the second cell culture medium during step (c) does not exceed 25 mM.

17. The method of any one of claims 1-16, wherein the density of oxygen (DO) in the first cell culture medium during steps (a) and/or (b) is maintained above 50%.

18. The method of any one of claims 1-17, wherein density of oxygen (DO) in the second cell culture medium during step (c) is maintained above 50%.

19. The method of any one of claims 1-18, wherein the fixed bed has a bed height of 2 cm or 10 cm.

20. The method of any one of claims 1-19, wherein the macrocarrier is a microfiber matrix.

21. The method of any one of claims 1-20, wherein the macrocarrier has a porosity between 60% and 99%.

22. The method of claim 21, wherein the macrocarrier has a surface area accessible to the cell of between 150 cm^2/cm^3 and 1000 cm^2/cm^3.

23. The method of any one of claims 1-22, wherein at least 5.0×10^7 TCID50/mL of the Enterovirus A virus is harvested in step (d).

24. The method of any one of claims 1-23, further comprising inactivating the Enterovirus A with one or more of beta-propiolactone (BPL), formalin, or binary ethylenimine (BEI).

25. The method of any one of claims 1-24, wherein the Enterovirus A virus is an attenuated virus.