[0005] Other vaccines have been developed by inactivating viruses after growth in cell culture. These "killed virus" vaccines induce immune responses due to the presence of high concentrations of antigen present. Examples of effective killed viral vaccines include, but are not limited to, vaccine for rabies, influenza, hepatitis A, and poliovirus.

[0006] Flaviviruses cause a number of human and animal diseases of significant impact. They are enveloped viruses with a RNA genome of approximately 11,000 bases. Most of the flaviviruses are transmitted by an arthropod vector, commonly mosquitoes. There are over 70 different flaviviruses that are grouped into three major categories based on serology: the dengue group, the Japanese encephalitis group and the yellow fever group. Expanding urbanization, worldwide travel and environmental changes (such as deforestation or rain patterns) have lead to the emergence of several flaviviruses as threats to human public health. Such viruses include, but are not limited to, yellow fever virus, the dengue viruses, West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis viruses.

[0007] Both live, attenuated viral vaccines and killed virus vaccines have been developed that are safe and protect against flavivirus diseases, for example, yellow fever and Japanese encephalitis.

Summary

[0008] Embodiments of this application generally relate to methods, compositions and uses for inducing, enhancing and accelerating viral growth. In certain embodiments, this application reports methods, compositions and uses of copolymer compositions for inducing accelerated viral growth and/or increasing viral plaque size. In other embodiments, methods, compositions and uses of copolymer compositions are reported for accelerating flaviviral growth, reducing flaviviral lag time and/or increasing flaviviral plaque size.

[0009] One limitation for producing vaccines has been large-scale manufacture and in vitro growth of the viruses to support the demand of vaccines. Thus, one of the needs that exist in the art is for enhancing and accelerating viral growth. Certain embodiments of the present disclosure concern methods and compositions for enhancing and accelerating viral growth. These compositions are of use, for example, in production of viral vaccines and viral byproducts of use in other technologies such as manufacturing of viral-related gene therapies and other viral products. In addition, embodiments herein may be of use to enhance or accelerate growth of viral cultures of use in killed virus vaccines.

[0009a] In one aspect, there is provided a method for manufacturing viruses, the method comprising:

growing host cells;

introducing to the host cells a composition comprising media for growing viral cultures comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers before, during, or after viral infection of the host cells; the one or more EO-PO block copolymers comprising poloxamer 407, poloxamer 403, or a combination thereof, wherein the concentration of the one or more EO-PO block copolymers is from 0.001% to 3.0%(w/v);

introducing viruses to the host cells before, during or after introduction of the composition;

incubating the host cells, the viruses, and the media for a predetermined period;

separating the media from the host cells; and

harvesting the viruses from the media.

[0009b] In another aspect, there is provided a method for manufacturing flaviviruses and/or dengue viruses, the method comprising:

growing host cells to near confluency;

introducing to the host cells a composition comprising media for growing the viral cultures and one or more ethylene oxide propylene oxide (EO-PO) block copolymers before, during, or after viral infection of the host cells; the one or more EO-PO block copolymers comprising poloxamer 407, poloxamer 403, or a combination thereof, wherein the concentration of the one or more EO-PO block copolymers is from 0.001% to 3.0% (w/v);

introducing the flaviviruses and/or dengue viruses to the host cells before, during or after introduction of the composition;

incubating the host cells, the flaviviruses and/or dengue viruses, and the growth media;

separating the media from the host cells; and

harvesting the flaviviruses and/or dengue viruses from the media.

[0009c] In another aspect, there is provided a composition for growing flaviviruses and/or dengue viruses comprising:

a flavivirus and/or a dengue virus culture;

one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprising poloxamer 407, poloxamer 403, or a combination thereof, wherein the concentration of the EO-PO block copolymer is from 0.063% to 3.0% (w/v);

a host cell culture; and

growth media, wherein the EO-PO block copolymers enhance growth for large scale manufacturing of the flaviviruses and/or a dengue virus in the host cell culture.

[0010] Certain compositions disclosed herein can include copolymers alone or in combination with other agents or compounds for enhancing and accelerating viral growth. Other embodiments herein concern combinations of excipients that enhance growth of live attenuated viruses. Copolymers of use herein include, but are not limited to, Pluronic F127, Pluronic F68, Pluronic P123, Pluronic P85, other polyethylene oxide-polypropylene oxide (EO-PO) block copolymers of greater than 3,000-4,000 MW or combinations thereof.

[0011] In accordance with these embodiments, viruses can include, but are not limited to, Flavivirus, Togavirus, Coronavirus, Rhabdovirus, Filovirus, Paramyxovirus, Orthomyxovirus, Bunyavirus, Arenavirus, Retrovirus, Hepadnavirus, Pestivirus, Picornavirus, Calicivirus, Reovirus, Parvovirus, Papovavirus, Adenovirus, Herpes virus, and Poxvirus. Some embodiments, directed to compositions of use in viral cultures, can include, but are not limited to, cultures having one or more viruses, such as a mixture of viral species or a single species, or one or more live, attenuated viruses grown in one or more copolymer compositions alone, or in combination with other agents.

[0012] In other embodiments, compositions contemplated herein can increase plaque size in a reduced or similar time period of growth, compared to a control culture without the disclosed composition, for use in tittering, manufacturing or measuring the activity of virus preparations. In some embodiments of the present disclosure, higher viral titers may be obtained in reduced time periods. Alternatively, compositions contemplated herein can reduce lag time or accelerate growth time up to several days compared to control viral cultures not using compositions contemplated herein.

[0013] Other embodiments concern virus populations for use in formulations and methods directed to vaccine formulations capable of reducing or preventing onset of a medical condition caused by one or more of the viruses contemplated herein. In accordance with these embodiments, medical conditions may include, but are not limited to conditions and/or infections including West Nile, dengue fever, Japanese encephalitis, Kyasanur forest disease, Murray valley encephalitis in Australia and New Guinea, Kunjin virus (a relative of West Nile), Alkhurma hemorrhagic fever, St. Louis encephalitis, hepatitis C virus infection, tick-borne encephalitis, yellow fever, the Usutu, Koutango, Yaonde viruses in Africa, and Cacipacore in South America. In certain embodiments, production time for generating vaccine formulations may be reduced by using compositions contemplated herein for accelerating viral growth production and manufacture, reducing lag time and/or increasing plague size of viral populations.

What is claimed is:

1. A method for manufacturing viruses, the method comprising:

growing host cells;

introducing to the host cells a composition comprising media for growing viral cultures comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers before, during, or after viral infection of the host cells; the one or more EO-PO block copolymers comprising poloxamer 407, poloxamer 403, or a combination thereof, wherein the concentration of the one or more EO-PO block copolymers is from 0.001% to 3.0%(w/v);

introducing viruses to the host cells before, during or after introduction of the composition;

incubating the host cells, the viruses, and the media for a predetermined period;

separating the media from the host cells; and

harvesting the viruses from the media.

2. The method of claim 1, wherein separating the media from the host cells further comprises removing aliquots of the media from the culture and introducing fresh media to the culture for further viral expansion.

3. The method of claim 1, further comprising increasing the growth of the viruses and/or increasing plague size of the cultures compared to viral population growth in cultures without the one or more EO-PO block copolymers.

4. The method of claim 1, wherein the growth media containing the viruses is removed harvesting the viruses from the growth media.

5. The method of claim 1, wherein at least one of the one or more EO-PO block copolymers comprises poloxamer 407.

6. The method of claim 1, wherein the media for growth comprises Dulbecco's Modified Eagle Medium (DMEM).

7. The method of claim 1, wherein the viruses comprise live, attenuated viruses for use in vaccines.

8. The method of claim 1, wherein the viruses are selected from the group consisting of Flavivirus, Togavirus, Coronavirus, Filovirus, Paramyxovirus, Orthomyxovirus, Bunyavirus, Arenavirus, Retrovirus, Hepadnavirus, Pestivirus, Herpes virus, and Poxvirus.

9. The method of claim 1, wherein the viruses are flavivirus viruses.

10. The method of claim 1, wherein the viruses are Poxvirus viruses.

11. The method of claim 1, wherein when harvested, the viral cultures have a titer between about $1x10^6$ pfu/ml to about $1x10^8$ pfu/ml.

12. The method of claim 1, wherein the host cells are in monolayers.

13. The method of claim 1, wherein the host cells comprise host cells grown to confluency at a density of about $1x10^4$ and about $1x10^5$ cells per cm^2.

14. The method of claim 1, wherein the host cells comprise Vero cells (African green monkey Vero cells), LLC-MK2 cells, or C6/36 mosquito cells.

15. The method of claim 1, wherein the concentration of the one or more EO-PO block copolymers is from 0.063% to 3.0%.

16. A method for manufacturing flaviviruses and/or dengue viruses, the method comprising:

growing host cells to near confluency;

introducing to the host cells a composition comprising media for growing the viral cultures and one or more ethylene oxide propylene oxide (EO-PO) block copolymers before, during, or after viral infection of the host cells; the one or more EO-PO block copolymers

comprising poloxamer 407, poloxamer 403, or a combination thereof, wherein the concentration of the one or more EO-PO block copolymers is from 0.001% to 3.0% (w/v);

introducing the flaviviruses and/or dengue viruses to the host cells before, during or after introduction of the composition;

incubating the host cells, the flaviviruses and/or dengue viruses, and the growth media;

separating the media from the host cells; and

harvesting the flaviviruses and/or dengue viruses from the media.

17. The method of claim 16, wherein the growth media containing the flaviviruses and/or dengue viruses is removed for harvesting the viruses from the growth media.

18. The method of claim 16, wherein the host cells comprise Vero cells (African green monkey Vero cells), LLC-MK2 cells, or C6/36 mosquito cells.

19. The method of claim 16, wherein the flaviviruses comprise dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus, or tick-borne encephalitis virus.

20. The method of claim 16, wherein the flaviviruses and/or dengue viruses comprise live, attenuated viruses.

21. The method of claim 20, wherein the live, attenuated viruses are chimeras.

22. The method of claim 16, wherein the growth media comprises Dulbecco's Modified Eagle Medium (DMEM).

23. The method of claim 16, wherein incubating comprises incubating for hours to several days.

24. The method of claim 16, wherein separating the media from the host cells further comprises removing aliquots of the media from the culture and introducing fresh media to the culture for further viral expansion.

25. A composition for growing flaviviruses and/or dengue viruses comprising:

a flavivirus and/or a dengue virus culture;

one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprising poloxamer 407, poloxamer 403, or a combination thereof, wherein the concentration of the EO-PO block copolymer is from 0.063% to 3.0% (w/v);

a host cell culture; and

growth media, wherein the EO-PO block copolymers enhance growth for large scale manufacturing of the flaviviruses and/or a dengue virus in the host cell culture.

26. The composition of claim 25, wherein the flavivirus and/or dengue virus cultures comprises live, attenuated flaviviruses.

27. The composition of claim 25, wherein the flavivirus and/or dengue virus culture comprises chimeric viruses.

28. The composition of claim 25, wherein the flavivirus cultures comprise West Nile viruses, yellow fever viruses, Japanese encephalitis viruses, St. Louis encephalitis viruses, or tick-borne encephalitis viruses.

29. The composition of claim 25, wherein the host cell culture comprises Vero cells (African green monkey Vero cells), LLC-MK2 cells, or C6/36 mosquito cells.

30. The composition of claim 25, wherein the growth media comprises Dulbecco's Modified Eagle Medium (DMEM).

31. The method of claims 1 or 16, wherein harvesting the viruses comprises removing aliquots of media to harvest the viruses.

32. The composition of claim 25, wherein EO-PO block copolymer comprises poloxamer 407.

33. The method of claim 1, wherein the viruses comprise enveloped viruses.