[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~~invention~~ 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 composition for growing viral cultures comprising: one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymer comprise poloxamer 407 (Pluronic F127), poloxamer 403 (Pluronic P123) or a combination thereof wherein the concentration of EO-PO block copolymer is about 0.001 % to about 3.0 %; a viral culture; a host cell; and a media, wherein the EO-PO block copolymers accelerate growth of viral cultures.

[0009b] In one aspect, there is provided a method for increasing viral growth rate comprising, administering to a host cell culture infected with a virus, a composition comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprise poloxamer 407 (Pluronic F127), poloxamer 403 (Pluronic P123) or a combination thereof, wherein the concentration of EO-PO block copolymer is about 0.001 % to about 3.0 %; and wherein the composition increases viral growth rate.

[0009c] In one aspect, there is provided a method for increasing viral growth rate comprising, administering a composition comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprise poloxamer 407 (Pluronic F127), poloxamer 403 (Pluronic P123) or a combination thereof, wherein the concentration of EO-PO block copolymer is about 0.001 % to about 3.0 %; to a host cell culture, before, during, or after viral infection of the host cell culture.

[0009d] In one aspect, there is provided a method for increasing plaque size of a viral culture comprising, administering to a host cell culture infected with a virus, a composition comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprise poloxamer 407 (Pluronic F127), poloxamer 403 (Pluronic P123) or a combination thereof wherein the concentration of EO-PO block copolymer is about 0.001 % to about 3.0 %; wherein the composition increases viral plaque size compared to a control viral culture without administering one or more EO-PO block copolymers.

[0009e] In one aspect, there is provided a method for reducing growth lag time of a viral culture comprising, administering to a host cell culture infected with a virus, a composition comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprise poloxamer 407 (Pluronic F127), poloxamer 403 (Pluronic P123) or a combination thereof wherein the concentration of EO-PO block copolymer is about 0.001 % to about 3.0 %; wherein the composition reduces lag time of the viral cultures compared to a control viral culture without administering one or more ethylene oxide propylene oxide (EO-PO) block copolymers.

[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 embodiment~~aspects~~ of the present disclosure~~invention~~, 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.

[0014] In certain embodiments, viral cultures contemplated for production herein may be used in compositions including, but not limited to, partially or wholly dehydrated or hydrated vaccine formulations or other viral formulations.

[0015] In certain embodiments, a live attenuated virus for use in a vaccine composition contemplated herein may include, but is not limited to, one or more live, attenuated flavivirus vaccines, including but not limited to, attenuated yellow fever viruses (*e.g.* 17D), attenuated Japanese encephalitis viruses, (*e.g.* SA 14-14-2), attenuated dengue viruses (*e.g.* DEN-2/PDK-53 or DEN-4Δ30), attenuated chimeric West Nile vaccines, or recombinant chimeric flaviviruses.

[0015a] In one aspect, there is provided a kit for culturing viruses comprising; at least one container; a composition comprising one or more ethylene oxide propylene oxide (EO-PO) block copolymers, the EO-PO block copolymers comprise poloxamer 407 (Pluronic F127), poloxamer 403 (Pluronic P123) or a combination thereof wherein the concentration of EO-PO block copolymer is about 0.001 % to about 3.0 %; and one or more viral cultures.

[0016] Other embodiments concern kits for growing viral cultures contemplated herein. It is contemplated that a kit may include partially or wholly dehydrated viral cultures of use in generating live, attenuated virus populations for vaccine production or other viral composition uses. It is also contemplated that a kit may include one or more growth inducing compositions disclosed herein.

Brief Description of the Drawings

[0017] The following drawings form part of the present specification and are included to further demonstrate certain embodiments herein. The embodiments may be better understood by reference to one or more of these drawings alone or in combination with the detailed description of specific embodiments presented.

[0018] Fig. 1 represents an exemplary graph of pluronic effects on viral growth after cellular infection and introduction of a control agent or copolymer composition during viral adsorption.

[0019] Fig. 2 represents an exemplary graph illustrating growth of viral cultures in the presence of a copolymer during viral adsorption and/or growth.

[0020] Fig. 3 represents an exemplary graph illustrating growth of viral cultures in the presence of increasing amounts of a copolymer-containing composition during viral adsorption and growth.

[0021] Fig. 4 represents an exemplary graph illustrating growth of viral cultures in the presence or absence of a specific copolymer added during viral adsorption.

[0022] Fig. 5 represents an exemplary table illustrating change in plague size of exemplary viral cultures in the presence or absence of various concentrations of copolymer.

Definitions

[0023] As used herein, "a" or "an" may mean one or more than one of an item.