NANOCAPSULES CARRYING CHIKUNGUNYA-ASSOCIATED PEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application No. 10201500725U, filed 29 January 2015, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of nanotechnology. In particular, the present invention relates to nanocapsules for peptide delivery.

BACKGROUND OF THE INVENTION

[0003] Chikungunya virus (CHIKV) is a human pathogen and one of the leading causes of mosquito-borne arthralgia in parts of Africa, India and Southeast Asia. The disease of the same name, Chikungunya is a transmitted by Chikungunya virus (CHIKV)-carrying Aedes mosquitoes. Since Chikungunya symptoms are very similar to Dengue fever, it is often misdiagnosed. In the year 2006, total number of 1,390,322 suspected Chikungunya fever cases was reported in India. The disease was unknown in Singapore until 2008, when it experienced its first successive waves of Chikungunya fever. According to Singapore Ministry of Health and The Strait Times, the total number of infections in the last five years is about 1500. With no specific treatment or any licensed vaccine to prevent Chikungunya, the disease remains a public health threat.

[0004] Therefore, there is a need to develop an effective vaccine against the disease caused by the Chikungunya virus.

SUMMARY

[0005] . In one aspect, there is disclosed a composition comprising a viral protein or fragment thereof, wherein the viral protein, or fragment thereof is enclosed within a nanocapsule, and wherein the viral protein, or fragment thereof is selected from a virus of the Togaviridae family, wherein the nanocapsule is formed by a scaffold protein, wherein the scaffold protein is a mutated ferritin protein encoded by SEQ ID NO. 13 or SEQ ID NO. 14.

[0006] In one example, there is disclosed a method of eliciting an immune response in a subject, the method comprising administering to the subject the composition as described herein.

[0007] In another example, there is dsiclosed a vaccine for use preventing an infection caused by an agent selected from the group consisting of Chikungunya virus, O'nyong'nyong virus, Semliki Forest virus, Sindbis virus, Aura virus, Babanki virus, Barmah Forest virus, Bebaru virus, Buggy Creek virus, Cabassou virus, Eastern equine encephalitis virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus, Kyzykagach virus, Mayaro virus, Middleburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, Ockelbo virus, Paramana virus, Pixuna virus, Rio Negro virus, Tonate virus, Trocara virus, Sagiyama virus, sleeping disease virus, Salmon pancreatic disease virus, Southern elephant seal virus, Una virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus, Whataroa virus and Ross River virusChikungunya virus, O'nyong'nyong virus, Semliki Forest virus, Sindbis virus, and Ross River virus, the vaccine comprising the composition as described herein.

[0008] In yet another example, there is disclosed a method of preventing or ameliorating an infection caused by an agent selected from the group consisting of Chikungunya virus, O'nyong'nyong virus, Semliki Forest virus, Sindbis virus, Aura virus, Babanki virus, Barmah Forest virus, Bebaru virus, Buggy Creek virus, Cabassou virus, Eastern equine encephalitis virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus, Kyzykagach virus, Mayaro virus, Middleburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, Ockelbo virus, Paramana virus, Pixuna virus, Rio Negro virus, Tonate virus, Trocara virus, Sagiyama virus, sleeping disease virus, Salmon pancreatic disease virus, Southern elephant seal virus, Una virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus, Whataroa virus and Ross River virusChikungunya virus, O'nyong'nyong virus, Semliki Forest virus, Sindbis virus and Ross River virus, the method comprising administering to a subject the composition as described herein.

[0009] In a further aspect, the present invention refers to a nucleic acid sequence comprising a gene encoding a viral protein or fragment thereof, a linker sequence and a scaffold protein ; wherein the viral protein, or fragment thereof is enclosed within a nanocapsule, and wherein the viral protein, or fragment thereof is selected from a virus of the Togaviridae family, wherein the nanocapsule is formed by a scaffold protein, wherein the scaffold protein is a mutated ferritin protein encoded by SEQ ID NO. 13 or SEQ ID NO. 14.

[0010] In yet a further aspect, the present invention refers to a vector comprising the nucleic acid sequence as described herein.

[0011] In one aspect, the present invention refers to an isolated host cell comprising the vector as described herein.

[0012] In another example, there is disclosed a method of producing the composition as described herein, the method comprising isolating the protein expressed by the vector as described herein, and adding a catalyst in an amount sufficient to catalyse the formation of the nanocapsule.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0014] **Fig. 1** shows a schematic showing the gene domain arrangement for the Chikungunya virus composition/vaccine, comprising an epitope, for example a CHIKV epitope, a linker sequence, for example SGG and a scaffold protein, for example ferritin. NdeI, NHeI, and BamHI are the restriction sites within or at the end of the sequence.

[0015] **Fig. 2** shows a schematic protein modelling image of the viral Chikungunya glycoprotein E2, showing the spatial orientation of various protein structures, as well as the E2EP3 epitope.

[0016] **Fig. 3** show a schematic depiction of the quaternary protein structures formed by (A) wild type (AfFtn) and (B) mutant (AfFtn-AA) *Archaeoglobulus fulgidus* ferritins, respectively. Wild type ferritin (AfFtn; SEQ ID NO: 15 or SEQ ID NO: 16) has a unique tetracosameric structure with triangular opening (~45 Å in diameter) for every six subunits, while the mutated ferritin AfFtn-AA is a mutated protein from the wild type where amino acid residues Lysine at site 150 and Arginine at site 151 were both replaced with Alanine. As a result of mutation, the AfFtn-AA has a closed spherical structure.

[0017] **Fig. 4** shows a general schematic outlining the mechanism underlying site-directed mutagenesis.

[0018] **Fig. 5** shows a schematic image of the *Archaeoglobulus fulgidus* ferritin proteins. The N-termini of the individual subunits have been marked in darker shading. Upon successful subunit production and subsequent 24-mer assembly, the attached viral epitopes should be protruding out from the ferritin structure from the N-terminus of each subunit.

Claims

1. A composition comprising a viral protein or fragment thereof, wherein the viral protein, or fragment thereof is enclosed within a nanocapsule, wherein the viral protein or fragment thereof is selected from a virus of the Togaviridae family, wherein the nanocapsule is formed by a scaffold protein, wherein the scaffold protein is a mutated ferritin protein encoded by SEQ ID NO. 13 or SEQ ID NO. 14.

2. The composition of claim 1, wherein the viral protein, or the fragment thereof, is selected from the group consisting of alphavirus and Rubivirus.

3. The composition of claim 2, wherein the alphavirus is selected from the group consisting of Chikungunya virus, O'nyong'nyong virus, Semliki Forest virus, Sindbis virus, Aura virus, Babanki virus, Barmah Forest virus, Bebaru virus, Buggy Creek virus, Cabassou virus, Eastern equine encephalitis virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus, Kyzykagach virus, Mayaro virus, Middleburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, Ockelbo virus, Paramana virus, Pixuna virus, Rio Negro virus, Tonate virus, Trocara virus, Sagiyama virus, sleeping disease virus, Salmon pancreatic disease virus, Southern elephant seal virus, Una virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus, Whataroa virus and Ross River virus.

4. The composition of claim 3, wherein the alphavirus is selected from the group consisting of Chikungunya virus, O'nyong'nyong virus, Semliki Forest virus, Sinbis virus, and Ross River virus.

5. The composition of claim 4, wherein the alphavirus is Chikungunya virus.

6. The composition of claim 1, wherein the viral protein or fragment thereof is an epitope of the Chikungunya virus.

7. The composition of claim 1, wherein the viral protein or fragment thereof has an identity of at least about 70%, about 80%, about 90%, about 92%, about 95%, about 97%, about 98% or about 99% of the viral protein or fragment thereof selected from

the group consisting of E2EP3 (encoded by SEQ ID NO: 1), E2EP4 (encoded by SEQ ID NO: 3), E2EP5 (encoded by SEQ ID NO: 4), , and CV (SEQ ID NO: 12); or wherein the viral protein or fragment thereof is selected from the group consisting of E2EP3 (encoded by SEQ ID NO: 1), E2EP3 mutant (encoded by SEQ ID NO: 2), E2EP4 (encoded by SEQ ID NO: 3), E2EP5 (encoded by SEQ ID NO: 4), E2EP5 mutant (encoded by SEQ ID NO: 5), and CV (encoded by SEQ ID NO: 6).

8. The composition of claim 1, wherein the viral protein or fragment thereof differs by at least 1, at least 2 or at least 3 amino acids from the viral protein or fragment thereof selected from the group consisting of E2EP3 (SEQ ID NO: 7), E2EP4 (SEQ ID NO: 9), E2EP5 (SEQ ID NO: 10), and CV (SEQ ID NO: 12); or wherein the viral protein or fragment thereof is selected from the group consisting of E2EP3 (SEQ ID NO: 7), E2EP3 mutant (SEQ ID NO: 8) E2EP4 (SEQ ID NO: 9), E2EP5 (SEQ ID NO: 10), E2EP5 mutant (SEQ ID NO: 11), and CV (SEQ ID NO: 12).

9. The composition of claim 1, wherein the nanocapsule comprises viral proteins or fragments thereof of one type only, or wherein the nanocapsule comprises 2, or 3, or 4 or more viral proteins or fragments thereof.

10. The composition of claim 1, wherein the nanocapsule is a nanocage.

11. The composition of claim 1, wherein the scaffold protein is a recombinant scaffold protein.

12. The composition of claim 1, wherein the viral protein or fragment thereof, is attached to the scaffold protein.

13. The composition of claim 12, wherein the viral protein, or fragment thereof, is attached to the scaffold protein via a linker sequence.

14. The composition of claim 13, wherein the linker sequence comprises the amino acids of the format $(G_XS_Y)_Z$; or wherein the linker sequence comprises the amino acids SGG.

15. The composition of claim 1, wherein the nanocapsule self-assembles.

16. A nucleic acid sequence comprising a gene encoding a viral protein or fragment thereof, a linker sequence and a scaffold; wherein the viral protein, or fragment thereof is enclosed within a nanocapsule, and wherein the viral protein, or fragment thereof is selected from a virus of the Togaviridae family, wherein the nanocapsule is formed by a scaffold protein, wherein the scaffold protein is a mutated ferritin protein encoded by SEQ ID NO. 13 or SEQ ID NO. 14.

17. A vector comprising the nucleic acid sequence according to claim 16.

18. An isolated host cell comprising the vector according to claim 17.