Claims

1. A method of preparing lipid vesicles, said method comprising dispersing a first liquid phase in a second liquid phase;

wherein said first liquid phase comprises a lipid phase and said second liquid phase comprises an aqueous phase; or said first liquid phase comprises an aqueous phase and said second liquid phase comprises a lipid phase;

said method comprising controlling provision of the first liquid phase in a first flow direction to a membrane, said membrane defining a plurality of pores; and controlling provision of the second liquid phase to the membrane in a crossflow to the first flow direction, via the plurality of pores, to form a lipid vesicle suspension.

2. The method according to claim 1 wherein the first liquid phase comprises a lipid phase and the second liquid phase comprises an aqueous phase.

3. The method according to claim 1 wherein the first liquid phase comprises an aqueous phase and the second liquid phase comprises a lipid phase.

4. The method according to any one of the previous claims wherein the lipid vesicles are liposomes or lipid nanoparticles (LNPs).

~~5. The method according to claim 4 wherein the lipid vesicles are liposomes.~~

~~6. The method according to claim 4 wherein the lipid vesicles are lipid nanoparticles (LNPs).~~

~~7~~5. A method of preparing lipid vesicles, said method comprising dispersing a first liquid phase in a second liquid phase, wherein said first liquid phase comprises a lipid phase;

wherein said method uses a crossflow emulsification apparatus; said crossflow emulsification apparatus (AXF) comprising:

an outer tubular sleeve provided with a first inlet at a first end; a lipid vesicle suspension outlet; and a second inlet, distal from and inclined relative to the first inlet;

a tubular membrane provided with a plurality of pores and adapted to be positioned inside the tubular sleeve; and

optionally an insert adapted to be located inside the tubular membrane, said insert comprising an inlet end and an outlet end, each of the inlet end and an outlet end being provided with chamfered region; the chamfered region is provided with a plurality of orifices and a furcation plate; and

controlling provision of the first liquid phase to the tubular membrane; and controlling provision of a second liquid phase to the tubular membrane via the plurality of pores to form a lipid vesicle suspension.

~~8. The method according to claim 7 wherein the first liquid phase comprises a lipid phase and the second liquid phase comprises an aqueous phase.~~

~~9. The method according to claim 7 wherein the first liquid phase comprises an aqueous phase and the second liquid phase comprises a lipid phase.~~

~~10~~6. The method according to ~~any one of claims 7 to 9~~ claim 7 wherein the lipid vesicles are liposomes or lipid nanoparticles (LNPs).

~~11. The method according to claim 10 wherein the lipid vesicles are liposomes.~~

~~12. The method according to claim 10 wherein the lipid vesicles are lipid nanoparticles (LNPs).~~

~~13~~7. The method according to any one of the preceding claims wherein the aqueous phase includes one or more active agents.

~~14~~8. The method according to any one of the preceding claims wherein the aqueous phase comprises a buffered solution.

~~15~~9. The method according to claim ~~14~~8 wherein the aqueous phase buffers include, but shall not be limited to, MES (2-(*N*-morpholino)ethanesulfonic acid), citrate, phosphate, acetate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), TRIS (tris(hydroxymethyl)aminomethane) and PBS (phosphate-buffered saline); and combinations thereof.

~~16~~10. The method according to any one of claims ~~7 to 15~~ 5 to 9 wherein when the one or more active agents is hydrophobic, then the one or more active agents may be included in the lipid phase.

~~17. The method according to any one of the preceding claims wherein the lipid phase includes one or more active agents.~~

~~18~~11. The method according to any one of the preceding claims wherein the lipid vesicles are produced unloaded and loaded afterwards (active loading).

~~19~~12. The method according to any one of claims 1 to ~~17~~10 wherein the lipid vesicles are produced loaded (passive loading).

~~20. The method according to claim 13 wherein the method produces lipid vesicles comprising of a lipid bilayer encapsulating an aqueous core and wherein the aqueous core includes one or more active agents.~~

~~21~~13. The method according to claim ~~13~~7 wherein the one or more active agents is a bioactive agents, such as a therapeutic agent (drug), vaccine and the like.

~~22~~14. The method according to claim ~~21~~13 wherein the bioactive agent is a therapeutic nucleic acid such as one encoding for an antigen.

~~23~~15. The method according to claim ~~22~~14 wherein therapeutic nucleic acids include, e.g., messenger RNA (mRNA), antisense oligonucleotides, ribozymes, DNAzymes, plasmids, immune stimulating nucleic acids, antagomir, antimir, mimic, supermir and aptamers.

~~24. The method according to claim 22 wherein the therapeutic nucleic acid is encodes for an antigen.~~

~~25. The method according to claim 22 wherein the lipid vesicles are LNPs.~~

~~26~~16. The method according to ~~claim 25~~ claim 1 wherein the lipid vesicles are LNPs and the LNPs are ionisable or cationic LNPs.

~~27~~17. The method according to any one of claims 1 to ~~26~~16 wherein the lipid vesicles comprise cationic lipid vesicles, such as, DDA (dimethyl dioctadecyl ammonium bromide) or DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) may suitably be used.

~~28. The method according to any one of claims 1 to 25 wherein the lipid vesicles comprise neutral lipid vesicles, optionally along with positively or negatively charged lipids; and any combination thereof.~~

~~29~~18. The method according to ~~claim 28~~ claim 1 wherein the lipid vesicles comprise neutral lipid vesicles and the neutral lipid vesicles comprise sphingosylphosphorylcholine (SPC), L-α-hydrogenated phosphatidylcholine (HSPC), distearoylphosphatidylcholine (DSPC) and 1-palmitoyl-2-oleoylphosphatidylcholine, (POPC), and the like; and combinations thereof.

~~30. The method according to any one of the preceding claims wherein the lipid vesicles comprise a lipid having a pKa in the range of 5.0 to 7.6.~~

~~31~~19. The method according to ~~claim 30~~ claim 1 wherein the lipid vesicles comprise a lipid having a pKa in the range of 5.0 to 7.6 and the lipids include a tertiary amine.

~~32~~20. The method according to claim ~~31~~19 wherein the lipid vesicles comprise 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane or 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane. Another suitable lipid having a tertiary amine is 1,2-dioleyloxy-N,Ndimethyl-3-aminopropane.

~~33. The method according to any one of the preceding claims wherein the lipid vesicles are formed from a single lipid or from a mixture of lipids.~~

~~34. The method according to any one of the preceding claims wherein the lipid vesicles comprise LUVs with a diameter in the range of 50-220nm.~~

~~35. The method according to any one of the preceding claims wherein the lipid vesicles comprise phospholipids.~~

~~36~~21. The method according to any one of the preceding claims wherein the lipid vesicles comprise a pegylated lipid.

~~37~~22. The method according to claim ~~36~~21 wherein the pegylated lipids include, but shall not be limited to, 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), pegylated diacylglycerol (PEG-DAG), e.g. l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), a pegylated phosphatidylethanoloamine (PEG-PE), a PEG succinate diacylglycerol (PEG- S-DAG), e.g. 4-O-(2',3'-di(tetradecanoyloxy)propyl- 1 -O-(ώ-methoxy(polyethoxy)ethyl)butanedioate (PEG-S-DMG), a pegylated ceramide (PEG-cer), or a PEG dialkoxypropylcarbamate, e.g. ώ-methoxy(polyethoxy)ethyl-N-(2,3-

di(tetradecanoxy)propyl)carbamate, 2,3-di(tetradecanoxy)propyl-N-(ώ-methoxy(polyethoxy)ethyl)carbamate or distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol) 2000]; and combinations thereof.

~~38. The method according to any one of the preceding claims wherein the amount of lipid used to form lipid vesicles is in a range from about 0.01 g to about 0.5 g per dose of e.g. vaccine.~~

~~39. The method according to claim 34 wherein more than 90% of lipid vesicles are less than about 220 nm.~~

~~40~~23. A method according to claim ~~7~~5 wherein the apparatus includes an insert.

~~41. A method according to claim 7 wherein the apparatus does not include an insert.~~

~~42. A method according to claim 7 wherein the lipid vesicle outlet is generally at a second end of the tubular sleeve.~~

~~43. A method according to claim 7 wherein the lipid vesicle outlet is generally at a side branch of the tubular sleeve.~~

~~44. A method according to claims 7 wherein the first inlet is a continuous phase first inlet and the second inlet is a disperse phase inlet.~~

~~45. A method according to claims 7 wherein the first inlet is a disperse phase first inlet and the second inlet is a continuous phase inlet.~~

~~46. A method according to claim 7 wherein the tubular membrane is located centrally within the outer sleeve, such that the spacing between the membrane and the sleeve comprises an annulus, of equal or substantially equal dimensions at any point around the tubular membrane.~~

~~47. A method according to claim 46 wherein the spacing is from about 0.05 to about 10mm.~~

~~48. A method according to claim 40 wherein the insert is tapered.~~

~~49. A method according to claim 40 wherein the tubular membrane is located centrally within the outer sleeve, such that the spacing between the membrane and the insert comprises an annulus, of equal or substantially equal dimensions at any point around the insert.~~

~~50. A method according to claim 49 wherein the spacing is from about 0.05 to about 10mm.~~

~~51. A method according to claim 7 wherein the internal diameter of the tubular membrane is from about 1mm to about 10mm.~~

~~52~~24. A method according to claim ~~7~~5 wherein the crossflow apparatus comprises a plurality of tubular membranes.

~~53. A method according to claim 52 wherein each membrane has an insert located inside it.~~

~~54. A method according to claims 52 or 53 wherein a plurality of membranes is grouped as a cluster of membranes positioned alongside each other.~~

~~55. A method according to claim 7 wherein the inlet and outlet ends of the outer sleeve will generally be provided with a seal assembly.~~

~~56. A method according to claim 55 wherein the seal assembly on the inlet and outlet ends of the outer sleeve are the same.~~

~~57. A method according to claims 55 or 56 wherein the seal assembly comprises a tubular ferrule provided with a flange at each end; and wherein a first flange located at the end adjacent to the outer sleeve (when coupled) is provided with a circumferential internal recess which acts as a seat for an O-ring seal, wherein the O-ring seal allows a loose fit as the membrane slides through the O-rings.~~

~~58. A method according to claim 57 wherein the O-ring seal is adapted to be located around the end of the insert and within a recess in the outer sleeve.~~

~~59. A method according to claim 7 wherein the membrane pores are laser drilled.~~

~~60. A method according to claim 59 wherein the membrane pores are substantially uniform in pore diameter, pore shape and pore depth.~~

~~61. A method according to claim 60 wherein the membrane pores are generally uniformly spaced.~~

~~62. A method according to claims 59 or 60 wherein the pores have a diameter of from about 1 μm to about 200 μm.~~

~~63. A method according to any one of claims 59 to 62 wherein the shape of the pores is substantially tubular.~~

~~64. A method according to any one of claims 59 to 63 wherein the interpore distance is from about 1 μm to about 5,000 μm.~~

~~65. A method according to any one of claims 59 to 64 wherein the surface porosity of the membrane may be from about 0.001% to about 20% of the surface area of the membrane.~~

~~66. A method according to any one of claims 59 to 65 wherein the pores are in a patterned arrangement.~~

~~67. A method according to claim 66 wherein the patterned arrangement is a square, triangular, linear, circular or rectangular arrangement.~~

~~68. A method according to claim 67 wherein the patterned arrangement is a square arrangement.~~

~~69. A method according to claim 7 wherein the membrane comprises a material selected from glass; ceramic; metal; polymer/plastic or silicon.~~

~~70. A method according to claim 69 wherein the membrane comprises a metal.~~

~~71. A method according to claim 70 wherein the metal is stainless steel.~~

~~72. A method according to claim 7 wherein the furcation plate is a bi-furcation plate or a tri-furcation plate.~~

~~73. A method according to claim 72 wherein the furcation plate is a tri-furcation plate.~~

~~74. A method according to claims 72 or 73 wherein the number of orifices provided in the insert is from 2 to 6.~~

~~75. A method according to claim 74 wherein the number of orifices provided in the insert is three.~~

~~76. A method according to any one of claims 72 to 75 wherein the chamfered region on the insert comprises a shallow chamfer.~~

~~77. A method according to claims 7 to 76 wherein the membrane emulsification technique comprises the use of laminar flow.~~

~~78. A method according to claim 7 wherein the lipid vesicles thus obtained have a high reproducibility both in the encapsulation rate and in the particle size distribution (polydispersity).~~

~~79. A method according to claim 7 wherein the lipid vesicles have a polydispersity index of ≤0.3.~~

~~80~~25. A method according to claim ~~7~~5 wherein the apparatus is suitable for preparing lipid vesicles with a PDI of from about 0.02 to about 0.3.

~~81~~26. Lipid vesicles prepared by the method according to any one of claims 1 to ~~80~~25.

~~82. Lipid vesicles according to claim 81 wherein the lipid vesicles are liposomes or lipid nanoparticles (LNPs).~~

~~83. Lipid vesicles according to claim 82 wherein the lipid vesicles are liposomes.~~

~~84. Lipid vesicles according to claim 82 wherein the lipid vesicles are lipid nanoparticles (LNPs).~~

85. Lipid vesicles according to any one of claims 81 to 84 wherein the lipid vesicle includes an active agent.

86. Lipid vesicles according to claim 85 wherein the active agent is a nucleic acid.

87. Lipid vesicles according to claim 86 wherein the nucleic acid encodes for an antigen.

88. A composition comprising lipid vesicles according to any one of claims 81 to 87 and a pharmaceutically acceptable excipient, carrier or diluent.

89. A composition according to claim 88 wherein the composition is suitable for the delivery of anti-cancer, anti-fungal and anti-inflammatory therapies.

90. A composition according to claim 89 wherein the active agent in the composition is selected from doxorubicin (Doxil®), amphotericin (Ambisome®) and extended release morphine (DepoDur™).

91. A composition according to claim 90 wherein the active agent is doxorubicin (Doxil®).

92. A composition according to claim 90 wherein the active agent is amphotericin (Ambisome®).

93. A composition according to claim 90 wherein the active agent is extended

release morphine (DepoDur™).

94. A method of modulating the expression of a polypeptide by a cell, comprising providing to a cell, lipid vesicles according to claims 81 to 87.

95. A vaccine comprising lipid vesicles according to any one of claims 81 to 87 and an antigen associated with a disease or pathogen.

96. A vaccine according to claim 95 wherein the lipid vesicles are liposomes or lipid nanoparticles (LNPs).

97. A vaccine according to claim 96 wherein the lipid vesicles are liposomes.

98. A vaccine according to claim 96 wherein the lipid vesicles are lipid nanoparticles (LNPs).

99. A method, lipid vesicle, composition or vaccine as herein described with reference to the accompanying description.