

POLYMER-ENABLED DELIVERY OF PHARMACEUTICAL AGENTS**Related Application**

[001] This application claims convention priority from Australian patent application 2023902095, filed 30 June 2023. The content of AU'095 is incorporated herein by reference.

Field of the Invention

[002] The present invention relates to biologically-compatible polymers and the present inventors' surprising discovery that such polymers stabilise certain pharmaceutical agents for a period sufficient to allow for their administration to a subject via modes of delivery such as intravascular, intramuscular, subcutaneous, oral inhaled or intranasal

[003] The present invention further relates to a method of enabling the delivery of one or more pharmaceutically active agents and the use of the biologically-compatible polymer in the manufacture of a medicament for the delivery of one or more pharmaceutically active agents. Preferred embodiments relate to intranasal delivery.

[004] In another embodiment, the present invention relates to a kit for enabling the intravascular, intramuscular, subcutaneous, oral inhaled or intranasal delivery of one or more pharmaceutically active agents.

[005] The present invention is envisaged to be useful in providing an alternative means of administration for a vaccine or medicament otherwise restricted to intramuscular injectable delivery. It is surprisingly found that the stabilising effect of the polymer enables delivery of the one or more pharmaceutically-active agents to a subject wherein intravascular, intramuscular, subcutaneous, oral inhaled or intranasal administration may not have been previously viable.

[006] The present invention is envisaged to be useful in providing an alternative means for the formulation, stabilisation, manufacture and storage of vaccines or medicaments otherwise restricted to cold shipment, cold storage and complex manufacturing steps that may involve microfluidic devices or other costly steps.

[007] Although the present invention will be described hereinafter with reference to its preferred embodiment, it will be appreciated by those of skill in the art that the spirit and scope of the invention may be embodied in many other forms.

Background of the Invention

[008] Any discussion of the prior art throughout the specification should in no way be

considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[0009] The constant development of RNA-based actives, including but not limited to vaccines for infectious disease control and oncological purposes as well as the ongoing COVID-19 (SARS-CoV-2) pandemic has driven societal awareness of and interest in such technologies. Several COVID-19 vaccines, such as the Pfizer-BioNTech and Moderna vaccines, use RNA to stimulate an immune response. When introduced into human tissue, the vaccine contains either self-replicating RNA or messenger RNA (mRNA), which both cause cells to express the SARS-CoV-2 spike protein. This teaches the body how to identify and destroy the corresponding pathogen. RNA vaccines often use nucleoside-modified messenger RNA. The delivery of mRNA is achieved by a coformulation of the molecule into lipid nanoparticles (LNPs) which protect the RNA strands and help their absorption into the cells.

[0010] The stability of LNPs and the incorporated RNA component/s over the intended shelf life of the vaccine are critical to achieving the intended biological activity. This usually limits such vaccines to limited shelf life at specific temperature, cold-shipment and storage. Such strict requirements cause challenges in achieving a broader deployment of such technologies.

[0011] As noted, a preferred embodiment of the present invention relates to Applicant's proprietary polymers that may act to stabilise the active/s in an mRNA vaccine, preferably a COVID-19 vaccine, across conditions thereby enabling and supporting intranasal administration. Whereas the ensuing discussion focusses upon such embodiments, the skilled person will appreciate that within the context of the overall invention, these are merely exemplary.

[0012] All presently-approved coronavirus vaccines are administered by intramuscular injection. However, with injection not being preferred or tolerated amongst a significant proportion of the population, various other types of vaccine delivery methods have been studied for future coronavirus vaccines. One such mode is that of intranasal delivery.

[0013] Intranasal vaccines target mucosal immunity in the nasal mucosa which is a portal for viral entrance to the body. These vaccines are designed to stimulate nasal immune factors, such as IgA. In addition to inhibiting the virus, nasal vaccines provide ease of administration because no needles (*e.g.*, needle phobia) are involved. Nasal vaccines have been approved for influenza, but not as yet for COVID-19.

[0014] A recent publication by Carvalho (*Nature Medicine*, Vol.28, December 2022, pp.2439-2440) has pinpointed why mRNA vaccines may be incompatible with intranasal delivery. More needs to be known about the correlates of protection in mucosal immunity in order to

understand how, or even if, this will affect infection. Reactogenicity was mild or moderate. Antigen-specific mucosal antibody responses to intranasal vaccination were detectable in a minority of participants, rarely exceeding levels seen after SARS-CoV-2 infection. Systemic responses to intranasal vaccination were typically weaker than after intramuscular vaccination with ChAdOx1 nCoV-19. Antigen-specific mucosal antibody was detectable in participants who received an intramuscular mRNA vaccine after intranasal vaccination. Seven participants developed symptomatic SARS-CoV-2 infection. In sum, this suggests that the formulation of intranasal ChAdOx1 nCoV-19 showed an acceptable tolerability profile but induced neither a consistent mucosal antibody response nor a strong systemic response.

[0015] Accordingly, the search for either a COVID-19 vaccine suitable for intranasal administration, or for a delivery vehicle that stabilises the active component/s prior to administration of existing vaccines or future formulation, or for a delivery system that retain the active component/s over a wide surface area of nasal mucosa to initiate and achieve antibody responses continues apace.

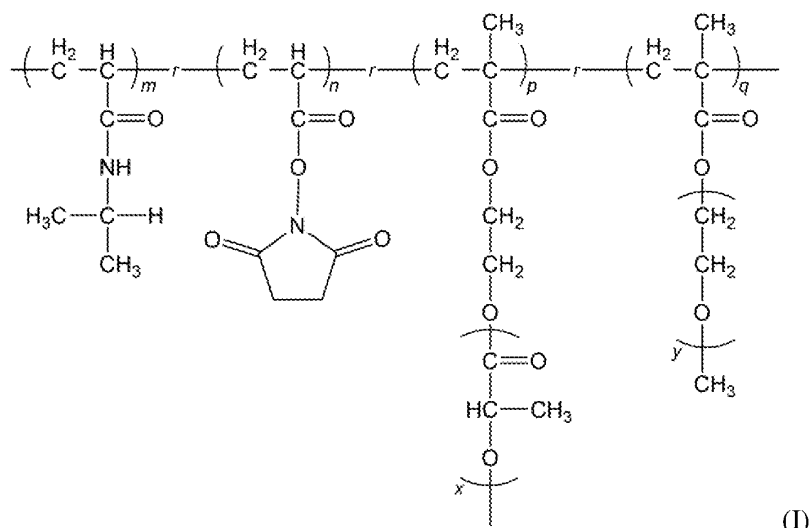
[0016] In a different sector of the biomedical field, the present Applicant, Trimph IP Pty Ltd, of Sydney, Australia, has been active over the past decade in patenting a suite of biocompatible polymers for medical applications. All of the patents and patent publications referred to herein are incorporated by reference in their respective entireties.

[0017] WO 2013/091001 (PCT/AU2012/001566) relates to polymers, especially polymers useful as hydrogels, and to the use of hydrogels for repair or restoration of tissue. In particular, the polymers and hydrogels of WO'001 can be used for the repair or restoration of cartilage, especially articular cartilage. The polymers comprise at least a monomer for binding water, a monomer for imparting mechanical properties and a monomer for binding to an extracellular protein. The hydrogels comprise a polymer comprising at least a monomer for binding water and a monomer for binding to an extracellular protein. Crosslinking polymers by binding of the extra-cellular matrix protein forms hydrogels.

[0018] A preferred polymer disclosed in WO'001 is Poly(NIPAAm-*co*-NAS-*co*-(PLA/HEMA)-*co*-OEGMA), *i.e.*, "PNPHO". The polymer PNPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, NAS in an amount of up to 15 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%. For certainty, the percentages recited relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

[0019] A preferred form of the polymer PNPHO is a polymer of Formula (I), as drawn below.

In addition, x is in the range of 1-1000 and y is in the range of 1-1000 and m, n, p, and q are in the range of 1-20. A person skilled in the art will be aware that the monomers A, B, C and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.



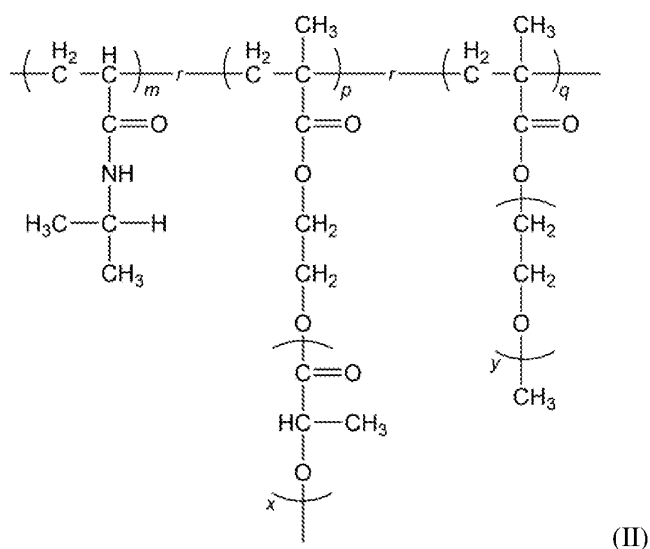
[0020] WO 2017/035587 (PCT/AU2016/050817) discloses biocompatible materials useful for tissue regeneration and repair, wherein the bioactive polymer may be in the form of a hydrogel, for example a thermoresponsive hydrogel. The bioactive polymer and resulting hydrogel of WO'587 may be used for the regeneration of bone tissue. Accordingly, the reference teaches methods of treating a bone defect in a mammal, the methods comprising administering a therapeutically effective amount of a hydrogel formed by the bioactive polymer to the mammal to treat the bone defect.

[0021] WO 2017/015703 (PCT/AU2016/050653) discloses a polymer comprising at least one antiseptic/analgesic/anti-inflammatory monomeric unit in conjunction with at least three further monomeric units, the three further monomeric units eliciting properties selected from the group consisting of: temperature activation, water solubility, mechanical strength, protein/polysaccharide bonding capacity, and combinations thereof. In particular, WO'703 discloses a polymer, wherein the water-soluble monomeric unit is a hydrophilic ethylene glycol (OEGMA) moiety; the mechanical strength-conferring monomeric unit is polylactide-co-2-hydroxy-ethylmethyl acrylate (PLA/HEMA); the protein-reactive monomeric unit is an N-acryloxysuccinimide (NAS) moiety; and the thermosetting monomeric unit is an N- isopropyl

acrylamide (NIPAAm) moiety. The antiseptic/analgesic/anti-inflammatory monomeric unit comprises a methacrylic ester derivative of salicylic acid (5-HMA or 4-HMA, or a combination thereof).

[0022] WO 2021/119727 (PCT/AU2020/051332) teaches a composition comprising a polymer and a natural or synthetic peptide or protein (NSPP) as Thymosin beta-4. The polymer comprises a first monomer for binding water, a second monomer for imparting mechanical properties, a third monomer for binding to an NSPP and a fourth monomer for imparting phase-transition behaviour. In particular, the composition forms an adhesive and flowable hydrogel upon administration into the body or onto the body surface, thereby assists in tissue repair and regeneration. Accordingly, WO'727 discloses methods of tissue repair and/or regeneration, the methods comprising administering the compositions by injection or by administering an aerosol, thereby to form a hydrogel at the body temperature of a mammal.

[0023] Finally, WO 2023/201397 (PCT/AU2023/050329) discloses a new polymer, "PPHO", *i.e.*, Poly(N-isopropylacrylamide-*co*-(polylactide/2-hydroxy methacrylate)-*co*-(oligo (ethylene glycol) / Poly(NIPAAm-*co*-(PLA/HEMA)-*co*-OEGMA)).



[0024] The polymer PPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%. In preferred embodiments, PPHO comprises OEGMA in about 1 to 15 mol% and/or PLA/HEMA in about 15 to 50 mol% and/or NIPAAm in an amount

of about 50 to 85 mol%. As above, the percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer. A representative polymer of PPHO (Formula (II)) is shown above.

[0025] It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

[0026] There is a general need in the art for effective intranasal delivery vehicles that may stabilise mRNA and/or LNPs for a sufficient period prior to their administration or to retain the formulation throughout the upper respiratory region to enable their intranasal administration.

[0027] It is against this background that the present invention has been developed. Various embodiments of the present invention may find utility relating to one or more of the general needs identified above.

[0028] In particular, the present invention is useful in providing a physical and commercial alternative to intramuscular injectable delivery of certain pharmaceutical substances such as COVID-19 vaccines.

[0029] Although the invention will be described with reference to specific examples it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

Summary of the Invention

[0030] In a broad form, the present invention relates to an effective concentration of a polymer to encapsulate and stabilise one or more active pharmaceutical agents within a vaccine or medicament to increase the stability and shelf life of the active/s and if needed, to encapsulate and retain one or more active pharmaceutical agents within a vaccine or medicament otherwise restricted mainly to intramuscular injectable delivery. It is surprisingly found that the polymer enables stabilisation of one or more active pharmaceutical agents to increase their shelf life and/or to allow their storage in a less restricted condition.

[0031] Applicant's polymer/s in solution or hydrogel form prior to administration can stabilise the active/s within a vaccine by preventing their agglomeration, *etc.*, so the shelf life of such vaccines can be extended and/or enhanced under relatively mild conditions (*e.g.*, ambient temperature). If then administered intranasally, it can adhere/retain the active agents throughout the upper respiratory region (nasal cavity) for better biological activity.

[0032] According to a first aspect of the present invention there is provided a polymer for forming a solution and/or hydrogel to stabilise one or more pharmaceutically active agents prior to, during or post-administration, the polymer comprising:

[0033] a first monomer for binding water;

- [0034] a second monomer for imparting mechanical properties to the scaffold;
- [0035] optionally, a third monomer for binding to a natural or synthetic peptide or protein (NSPP); and
- [0036] a fourth monomer for imparting phase-transition behaviour.
- [0037] In an embodiment, the stabilising of one or more pharmaceutically active agents prior to administration is to prevent their degradation, denaturation, sheering or any chemical or physical changes that can impact their biological activity.
- [0038] In an embodiment, the administration is by way of intravascular, intramuscular, subcutaneous, inhalable lung, oral inhaled or intranasal administration.
- [0039] In an embodiment, the administration is intranasal.
- [0040] In an embodiment, the first monomer is selected from: polyethers, polyvinyl alcohol (PVA); poly(vinyl pyrrolidone) (PVP); poly(amino acids) and dextran.
- [0041] In an embodiment, the polyethers are selected from: polyethylene glycol (PEG), oligo(ethylene glycol) (OEG), polyethylene oxide (PEO), polyethylene oxide-co-propylene oxide (PPO), co-polyethylene oxide block or random copolymers thereof.
- [0042] In an embodiment, the first monomer is oligo (ethylene) glycol monomethyl ether methacrylate (OEGMA).
- [0043] In an embodiment, the second monomer is a methacrylate, or a random co-polymer comprising a methacrylate.
- [0044] In an embodiment, the second monomer is selected from: hydroxyethyl methacrylate (HEMA), a hydroxyethyl methacrylate poly(lactic acid) copolymer (PLA/HEMA), poly(lactic acid), poly(caprolactone), poly(glycolide), poly(glycolide-colactide) or poly(glycolide-co-caprolactone).
- [0045] In an embodiment, the second monomer is hydroxyethyl methacrylate poly(lactic acid) (PLA/HEMA).
- [0046] In an embodiment, the third monomer has electrophilic functional groups for binding to the NSPP.
- [0047] In an embodiment, the third monomer is selected from: N-hydroxysulfosuccinimide (SNHS), N-hydroxyethoxylated succinimide (ENHS), and N-acryloxysuccinimide (NAS).
- [0048] In an embodiment, the third monomer is N-acryloxysuccinimide (NAS).
- [0049] In an embodiment, the fourth monomer has a lower critical solution temperature (LCST) less than about 37 °C.
- [0050] In an embodiment, the fourth monomer is selected from: poly(ethylene oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide) (PNIPAAm) homopolymers

and copolymers.

[0051] In an embodiment, the fourth monomer is (N-isopropylacrylamide) (NIPAAm).

[0052] In an embodiment, the polymer comprises the first monomer in an amount of from about 1 to about 15 mol%.

[0053] In an embodiment, the polymer comprises the second monomer in an amount of from about 5 to about 50 mol%.

[0054] In an embodiment, the polymer comprises the third monomer in an amount of from about 0 to about 15 mol%.

[0055] In an embodiment, the polymer comprises the fourth monomer in an amount of from about 50 to about 85 mol%.

[0056] In an embodiment, the polymer comprises: the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of 0 to about 15 mol%; and the fourth monomer in an amount which makes up the remainder to 100% of the polymer.

[0057] In an embodiment, the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm,

[0058] wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15 mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%.

[0059] In an embodiment, the one or more pharmaceutically-active agents comprise a therapeutic active, and/or vaccine otherwise restricted to delivery via intramuscular injection. Non-limiting examples include mRNA and siRNA vaccines and the like.

[0060] In an embodiment, the vaccine otherwise restricted to delivery via intramuscular injection is a COVID-19 (SARS-CoV-2) vaccine.

[0061] In an embodiment, the COVID-19 vaccine is the Pfizer-BioNTech (Comirnaty/Tozinameran) vaccine.

[0062] In an embodiment, the polymer is present in a concentration greater than or equal to about 15 mg/mL.

[0063] In an embodiment, the polymer is present in a concentration between about 25 and about 35 mg/mL.

[0064] In an embodiment, the polymer is present in a concentration of about 35 mg/mL.

[0065] In an embodiment, the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm;

[0066] wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15

mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%;

[0067] wherein the vaccine is an mRNA vaccine, preferably the Pfizer-BioNTech (Comirnaty/Tozinameran) COVID-19 vaccine;

[0068] wherein the polymer is present in a concentration of about 35 mg/mL; and

[0069] wherein the polymer preserves the integrity of the mRNA at 37 °C for up to 4 days.

[0070] Preferably, the polymer the polymer comprises:

[0071] a first monomer for binding water;

[0072] a second monomer for imparting mechanical properties to the polymer;

[0073] optionally, a third monomer for binding to a natural or synthetic peptide or protein (NSPP); and

[0074] a fourth monomer for imparting phase-transition behaviour.

[0075] In an embodiment, the first monomer is selected from: polyethers, polyvinyl alcohol (PVA); poly(vinyl pyrrolidone) (PVP); poly(amino acids) and dextran.

[0076] In an embodiment, the polyethers are selected from: polyethylene glycol (PEG), oligo(ethylene glycol) (OEG), polyethylene oxide (PEO), polyethylene oxide-co-propylene oxide (PPO), co-polyethylene oxide block or random copolymers thereof.

[0077] In an embodiment, the first monomer is oligo (ethylene) glycol monomethyl ether methacrylate (OEGMA).

[0078] In an embodiment, the second monomer is a methacrylate, or a random co-polymer comprising a methacrylate.

[0079] In an embodiment, the second monomer is selected from: hydroxyethyl methacrylate (HEMA), a hydroxyethyl methacrylate poly(lactic acid) copolymer (PLA/HEMA), poly(lactic acid), poly(caprolactone), poly(glycolide), poly(glycolide-colactide) or poly(glycolide-co-caprolactone).

[0080] In an embodiment, the second monomer is hydroxyethyl methacrylate poly(lactic acid) (PLA/HEMA).

[0081] In an embodiment, the third monomer has electrophilic functional groups for binding to the NSPP.

[0082] In an embodiment, the third monomer is selected from: N-hydroxysulfosuccinimide (SNHS), N-hydroxyethoxylated succinimide (ENHS), and N-acryloxysuccinimide (NAS).

[0083] In an embodiment, the third monomer is N-acryloxysuccinimide (NAS).

[0084] In an embodiment, the fourth monomer has a lower critical solution temperature (LCST) less than about 37 °C.

[0085] In an embodiment, the fourth monomer is selected from: poly(ethylene oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide) (PNIPAAm) homopolymers and copolymers.

[0086] In an embodiment, the fourth monomer is (N-isopropylacrylamide) (NIPAAm).

[0087] In an embodiment, the polymer comprises the first monomer in an amount of from about 1 to about 15 mol%.

[0088] In an embodiment, the polymer comprises the second monomer in an amount of from about 5 to about 50 mol%.

[0089] In an embodiment, the polymer comprises the third monomer in an amount of from about 0 to about 15 mol%.

[0090] In an embodiment, the polymer comprises the fourth monomer in an amount of from about 50 to about 85 mol%.

[0091] In an embodiment, the polymer comprises: the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of 0 to about 15 mol%; and the fourth monomer in an amount which makes up the remainder to 100% of the polymer.

[0092] In an embodiment, the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm, wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15 mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%.

[0093] In an embodiment, the polymer comprises: OEGMA in an amount of about 5 mol%, HEMA-PLA in an amount of about 7 mol%, NAS in an amount of greater than about 7 mol% and NIPAAm in an amount about 81 mol%.

[0094] According to a second aspect of the present invention there is provided a method for the delivery of one or more pharmaceutically active agents, the method comprising administration to a subject in need thereof an effective concentration of the one or more pharmaceutically active agents dispersed within a polymer as defined according to the first aspect of the invention.

[0095] In an embodiment, the administration is performed at ambient temperature, with the polymer transitioning to its hydrogel form at higher (*e.g.*, body) temperatures.

[0096] In an embodiment, the administration is performed via intranasal spray or deposition.

[0097] In an embodiment, the one or more pharmaceutically active ingredients may be dispersed within the polymer *in situ*, or pre-formulated and stored under conditions supporting

viability of the active/s.

[0098] In an embodiment, the conditions supporting viability of the active/s comprise freeze-drying the formulation prior to reconstitution of it.

[0099] According to a third aspect of the present invention there is provided use of a polymer as defined according to the first aspect of the present invention in the manufacture of a medicament for delivery of one or more pharmaceutically active agents.

[00100] In an embodiment, the administration is performed under ambient or substantially ambient conditions, and thereafter reaches body temperature whereupon the transition to hydrogel occurs.

[00101] In an embodiment, the administration is performed, via intravascular, intramuscular injection or intranasal delivery or any other routes of administration.

[00102] In an embodiment, the one or more pharmaceutically active ingredients may be dispersed within the polymer *in situ*, or pre-formulated and stored under conditions supporting viability of the active/s.

[00103] In an embodiment, the conditions supporting viability of the active/s comprise freeze-drying the formulation prior to reconstitution of it.

[00104] According to a fourth aspect of the present invention there is provided a kit for enabling delivery of one or more pharmaceutically active agents, the kit comprising a polymer as defined according to the first aspect of the present invention; an effective concentration of the one or more pharmaceutically active agents stored under appropriate conditions; and optionally instructions for adding an effective concentration of the polymer to the one or more pharmaceutically active agents.

[00105] In an embodiment, the kit further comprises means for effecting the intranasal delivery of the one or more pharmaceutically active agents and the effective concentration of the polymer.

[00106] In an embodiment, the kit comprises the polymer and the one or more pharmaceutically active agents in pre-mixed form.

Definitions and Nomenclature

[00107] In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one having

ordinary skill in the art to which the invention pertains.

[00108] “Stabilisation” of the one or more pharmaceutically-active agent/s is intended to cover the concept of “shelf-life stabilisation”. As such, by providing a stabilisation effect, the active agent/s’ storage conditions (*e.g.*, temperature) can be simplified and the vaccines stored for a longer period of time. This is mainly related pre-administration of the vaccine. Post-administration, the application of the polymer/vaccine, specifically from a stability and slow/controlled release perspectives is by way of intravascular, intramuscular, subcutaneous, oral inhaled, intranasal delivery, etc.

[00109] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

[00110] The terms “preferred” and “preferably” refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful and is not intended to exclude other embodiments from the scope of the invention.

[00111] As used herein a wording defining the limits of a range or length such as, for example, “from 1 to 5” means any integer from 1 to 5, *i.e.*, 1, 2, 3, 4 and 5. In other words, any range defined by two integers explicitly mentioned is meant to comprise and disclose any integer defining the limits and any integer comprised in the range.

[00112] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein are to be understood as modified in all instances by the term “about”. The examples are not intended to limit the scope of the invention. In what follows, or where otherwise indicated, “%” will mean “weight %”, “ratio” will mean “weight ratio” and “parts” will mean “weight parts”.

[00113] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard variations found in their respective testing measurements.

[00114] The present specification uses the following abbreviations:

mRNA	Messenger ribonucleic acid
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LNP	Lipid nanoparticles
ECM	Extracellular matrix
EHNS	N-hydroxyethoxylated succinimide
HEMA	Hydroxyethyl methacrylate
LA	Lactic acid
NAS	N-acryloxysuccinimide
NIPAAm	N-isopropylacrylamide
NSPP	Natural or synthetic peptide or protein
OEGMA	Oligo (ethylene) glycol monomethyl ether methacrylate
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
PEO	Polyethylene oxide
PLA/HEMA	Hydroxyethyl methacrylate poly(lactic acid)
PPO	Polyethylene oxide- <i>co</i> -propylene oxide
PVA	Polyvinyl alcohol
PVP	Poly(vinyl pyrrolidone)
PNPHO	Poly(N-isopropylacrylamide- <i>co</i> -(N-acryloxysuccinimide)- <i>co</i> -(polylactide/2-hydroxy methacrylate)- <i>co</i> -(oligo (ethylene glycol) / Poly(NIPAAm- <i>co</i> -NAS- <i>co</i> -(PLA/HEMA)- <i>co</i> -OEGMA)
PPHO	Poly(N-isopropylacrylamide- <i>co</i> -(polylactide/2-hydroxy methacrylate)- <i>co</i> -(oligo (ethylene glycol) / Poly(NIPAAm- <i>co</i> -(PLA/HEMA)- <i>co</i> -OEGMA)
SNHS	N-hydroxysulfosuccinimide
TB4	Thymosin beta-4 or Thymosin β -4
TPXXX	“TP” designates PNPHO or dialysed PNPHO; “XXX” represents the concentration of polymer, <i>e.g.</i> , TP050 = 50 mg/mL PNPHO in PBS.
TLXXX	“TL” designates PPHO or dialysed PPHO; “XXX” represents the concentration of polymer, <i>e.g.</i> , TL100 = 100 mg/mL PPHO in PBS.

Brief Description of the Drawings

[00115] Preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

[00116] *Figure 1* shows the stability at 4 °C of (a) formulations containing TP concentrations 005, 010 and 015 $\pm$ mRNA; (b) five-day stability study of mRNA integrity with

TP concentrations 0, 005, 010 and 015; and (c) five-day stability study of mRNA content with TP concentrations 0, 005, 010 and 015.

[00117] *Figure 2* shows a Zetasizer analysis of the particle sizes in the formulations stored at 4 °C on Day 1. Formulations of mRNA with and without PNPFO addition at concentrations of 5 mg/mL to form TP005 (a), 10 mg/mL to form TP010 (b) and 15 mg/mL to form TP015 (c).

[00118] *Figure 3* shows the stability at 37 °C of (a) formulations containing TP concentrations 0, 015 and 035 and mRNA with hydrogels formed (37 °C) and redissolved (after incubation at 4 °C) and mRNA integrity assessed for 24 h and 4 days ± aerosolising with VP7 Aptar nasal pump; (b) mRNA content measured for immediate (day 0) 24 h (day 1) and 96 h (Day 4) for PBS and TP50 formulations with the additional sampling of the supernatant for TP035.

[00119] *Figure 4* shows (a) nanoparticle formation ability of PNPFO polymer dialysed and non-dialysed at 25 mg/mL and 35 mg/mL concentration; (b) functional assay of mRNA-Lipofectamine with and without PNPFO addition at 25 mg/mL and 35 mg/mL of non-dialysed PNPFO (*Figure 4b-i*) and dialysed PNPFO (*Figure 4b-ii*).

[00120] *Figure 5* shows micelle formation wherein the concentration driven micelle formation potential of PNPFO polymer was assessed. To determine the critical micelle concentration (CMC) of PNPFO, serial dilutions of the PNPFO polymer from 25 mg/mL were performed.

[00121] *Figure 6* shows the complex coacervation of nanoparticles. Negatively-charged bovine serum albumin (BSA), a model therapeutic agent, was first added dropwise at a concentration of 1 mg/mL to PNPFO solution (0.5 mg/mL). Then, chitosan was used to create a nanoparticle by magnetically mixing chitosan (1 mg/mL; pre-dissolved in 1% acetic acid) and PNPFO at ratios of 1:5, 1:1 and 5:1 to understand the optimal polymer: polymer ratio for the formation of a nanocarrier.

[00122] *Figure 7* shows the relationship between the hydrodynamic diameter and PDI of the PNPFO polymer at various concentrations. In this study, the CMC of PNPFO polymer was reached at 0.5 mg/mL due to the initiation of self-aggregation of monomeric polymer. This was evident by the low PDI (< 0.5) at PNPFO concentrations of 0.5 mg/mL or above.

[00123] *Figure 8* shows the hydrodynamic diameter of blank and BSA-encapsulated NPs. As compared to blank NPs, the encapsulation of BSA as a model drug in PNPFO:Chitosan (1:1) formulation significantly reduced the particle size ($p < 0.05$) from 313.97 ± 43.71 nm to 112.41 ± 15.27 nm.

[00124] *Figure 9* shows the drug deposition pattern in silicon nasal cast. Over 20 min, an increasing amount of drug deposition was seen in the nasal cavity for the BSA-loaded PNPFO:Chitosan 1:1 formulations. In particular, the turbinate region demonstrated a relatively significant amount of drug deposition, as indicated by the bright pink colour of the Sar-Gel.

[00125] *Figure 10* shows the results of a qualitative study of nasal deposition using commercially available nasal cast at time points 0 and 20 min. The nasal cast deposition study using the BIVAX nasal spray device demonstrates that the TP035 formulation can be deposited region specifically (upper olfactory region) and that deposition is sustained for up to 20 min post deposition.

[00126] *Figure 11* shows the percentile size of particles with and without mRNA in samples TP007, TP015 and TP035 (a) and comparison of Z-ave (b) and Pdi (c).

[00127] *Figure 12* shows the integrity of mRNA at static condition (a) and after spray (b), stored at 37 °C for up to 4 days.

[00128] *Figure 13* shows mRNA and LNP (lipofectamine) separation and semi-quantification with GPC method (a) and preparation of different eGFP-mRNA/LNP formulations with/without TP or TL carrier. Control; mixture of mRNA with LNP, the standard formulation without the addition of TP (b-i), Formulation A with the carrier that involves mRNA/LNP mixing followed by the addition to TP solution (b-ii), Formulation B with the carrier that involves lipofectamine addition to TP carrier, followed by mRNA addition (b-iii), Formulation C with the carrier that involves mRNA dissolution directly in TP carrier, followed by the addition of LNP.

[00129] *Figure 14* shows gel electrophoresis bands of the carrier (no mRNA) (a) and with mRNA (b).

[00130] *Figure 15* shows DLS results for TP000 (control; (a)), TP035 (b), TP050 (c) and TP100 (d). Comparison of the particle size measurements (e) and Pdi of the particles (f) after 3 days of storage at 2-8 °C.

[00131] *Figure 16* shows mRNA encapsulation (a) and particle size measurements (b) of the control (no carrier; TP000) and TP035, TP050 and TP100 after 14 days of storage at 2-8 °C.

[00132] *Figure 17* shows e-GFP expression on epithelium cells (a-i) with eGFP-mRNA (a-ii), eGFP-mRNA + TP035 (a-iii) and eGFP-mRNA + TP035 + lipfectomine (a-iv). Live imaging of the cells at end point, 48 hours (b-i) and the fluorescence intensity at different time points (b-ii).

[00133] *Figure 18* shows nasal spray of TP000 (control; without the carrier system) in an *in vitro* human model (a), significant nose runoff (a-i and a-ii) and minimal coverage of upper

regions with the TP000 spray (a-iii). Nasal spray of TP035+ formulation (b), no nose runoff (b-i) and wide surface area coverage of nasal cavity after TP035+ spray (b-ii and b-iii).

[00134] *Figure 19* shows deposition patterns of TP000 (control) and TP035+ formulations 0, 5, 10, 15 and 20 minutes post-application (a). Volume retentions at the application site for TP000 and TP035+ formulations (b). In relation to volume retention, there are statistically significant differences ($p < 0.001$) at all time points between TP000 and TP035+. The surface area coverage of nasal cavity (c) after application. In relation to the surface area coverage, there are statistically significant differences ($p < 0.001$) for measurements between TP000 and TP035+ from 0 to 15 minutes; there was no statistical difference at $t=20$.

[00135] *Figure 20* shows the mRNA percentage encapsulation of formulations freshly made without the carrier (TP000) and with the carrier (TP025+) and the formulation made with the carrier, freeze-dried and reconstituted with water (TP025+FD).

Detailed Description of the Embodiments

[00136] The present invention will now be more fully described with reference to the accompanying examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

[00137] Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

[00138] One skilled in the art will recognise many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[00139] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

[00140] Disclosed broadly herein is the use of a biocompatible polymer for enabling the intranasal administration of a mRNA/LNP-based COVID-19 vaccine, particularly the Pfizer-BioNTech (Comirnaty) vaccine.

Polymers

[00141] The term “polymer”, as used herein, refers to a large molecule (macromolecule) composed of repeating structural units (monomers). These subunits are typically connected by covalent chemical bonds. Polymers can be linear or branched polymers. Preferably, the polymers of the present invention are copolymers comprising three or more different monomers. For example, in one embodiment, the polymers of the present are exemplified by Formulae (I) and (II) defined above.

[00142] The term “monomer”, as used herein, refers to a structural unit that can be combined to form a polymer, but that itself may also be a polymer, or a derivative of a monomer or polymer. Monomers of this type are herein also referred to as “macromonomers”. Herein a “macromonomer” is a polymer or oligomer the molecules of which each have one end-group that acts as a monomeric molecule, so that each polymer or oligomer molecule contributes only a single monomer unit to a chain of the product polymer.

[00143] The polymer of the present invention comprises: a first monomer for binding water; a second monomer for imparting mechanical properties to the polymer; an optional third monomer for binding to a natural or synthetic peptide or protein (NSPP); and a fourth monomer for imparting phase-transition behaviour.

First monomer: Water-binding monomer

[00144] As discussed above, the advantages of the polymer of the present invention can be attributed, at least in part, to the particular components that make up the polymers of the present invention. A particularly advantageous property of the polymers of the present invention is their water-binding capacity. The presence of water in the polymer of the present invention provides an environment that resembles both that of the natural environment of the damaged tissue (which assists in tissue regeneration) and the required compression resistance to the polymer.

[00145] Accordingly, the preferred polymers used herein should include monomers or units that are able to bind water to such a capacity that a malleable structure is able to form when the polymer is hydrated. In addition, the structure thus formed should have the required compression resistance and resilience.

[00146] A person skilled in the art will understand that water-binding monomers need to be present in the polymers of the present invention in proportions that are sufficient to produce a polymer that fulfils these requirements. Generally, the proportion of water-binding monomers

in the polymer is about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50 molar ratio of water binding:mechanical strength monomers. In fact, the water-binding monomers need to make the polymer not only hydrophilic, but impart much more significant water-binding capacities to the polymer. Accordingly, polymers in accordance with the present invention will have water-binding capacities of between about 70% and about 500%, between about 80% and about 400%, between about 90% and 300% or between about 100% and 200%. For example, the water-binding capacity of the polymers of the present invention is about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 210%, about 220%, about 230%, about 240%, about 250%, about 260%, about 270%, about 280%, about 290%, about 300%, about 310%, about 320%, about 330%, about 340%, about 350%, about 360%, about 370%, about 380%, about 390%, about 400%, about 410%, about 420%, about 430%, about 440%, about 450%, about 460%, about 470%, about 480%, about 490%, or about 500%.

[00147] Suitable examples of water-binding monomers include those that can be synthesised into polymers such as polyethers (*e.g.*, alkylene polyoxides such as polyethylene glycol (PEG), oligo(ethylene glycol) (OEG), polyethylene oxide (PEO), polyethylene oxide-co-propylene oxide (PPO), co-polyethylene oxide block or random copolymers, polyvinyl alcohol (PVA)), poly(vinyl pyrrolidinone) (PVP), poly(amino acids) and dextran. The polyethers, and more particularly oligo(oxyalkylenes) (*e.g.*, OEG), are especially preferred, because they have the requisite water-binding capacity, are simple to synthesise and/or purchase, and are inert, in the sense that they illicit minimal or no immune response from the tissues into which they are placed.

[00148] In addition, any of a variety of hydrophilic functionalities can be used to make a monomer (and therefore a polymer formed from such a monomer) water soluble. For example, functional groups like phosphate, sulphate, quaternary amine, hydroxyl, amine, sulfonate and carboxylate, which are water soluble, may be incorporated into a monomer to make it water soluble.

[00149] Monomers may also be reacted with other compounds to form “macromonomers”. Thus, the first monomer may optionally be a macromonomer.

[00150] A preferred first monomer which is a macromonomer is oligo(ethyleneglycol) monomethyl ether methacrylate (OEGMA), which is a hydrophilic monomer composed of two hydrophilic monomers: ethylene glycol and methacrylate.

[00151] Preferably, the polymer comprises the first monomer in an amount of from

about 1 to about 15 mol%. In various embodiments, the first monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. In various embodiments, the first monomer may be present from about 1 to about 15, about 2 to about 14, about 3 to about 13, about 4 to about 12, about 5 to about 11, about 6 to about 10, about 7 to about 9, or about 8 mol%.

Second monomer: Monomer imparting mechanical properties

[00152] As discussed above, the advantageous properties of the polymer of the present invention can be attributed, in part, to the particular components that make up the polymers of the present invention. In some embodiments, the polymers of the present invention are able to contribute additional mechanical properties and adhesivity to the polymer of the present invention.

[00153] A person skilled in the art will understand that monomers capable of imparting mechanical properties to a polymer need to be present in the polymers of the present invention in proportions that are sufficient to produce a polymer having the desired mechanical properties. Generally, the proportion of “mechanical” monomers in the polymer is about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50 molar ratio of water binding:mechanical strength monomers. Suitable examples of monomers that are capable of imparting mechanical properties (e.g. compression resistance) to a polymer include acrylates such as hydroxyethyl methacrylate (HEMA), polyesters such as poly(lactic acid), poly(caprolactone), poly(glycolide), and their random co-polymers (e.g. poly(glycolide-*co*-lactide) and poly(glycolide-*co*-caprolactone)).

[00154] Monomers may also be reacted with other compounds to form “macromonomers”. A preferred second monomer which is a macromonomer is hydroxyethyl methacrylate poly(lactic acid) (PLA/HEMA).

[00155] Preferably, the polymer comprises the second monomer in an amount of from about 1 to about 50 mol%. In various embodiments, the second monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 mol%. In various embodiments, the second monomer may be present from about 1 to about 15, about 2 to about 49, about 3 to about 48, about 4 to about 47, about 5 to

about 46, about 6 to about 45, about 7 to about 44, about 8 to about 43, about 9 to about 42, about 10 to about 41, about 11 to about 40, about 12 to about 39, about 13 to about 38, about 14 to about 37, about 15 to about 36, about 16 to about 35, about 17 to about 34, about 18 to about 33, about 19 to about 34, about 20 to about 33, about 21 to about 30, about 22 to about 29, about 23 to about 28, about 24 to about 27, or about 25 to about 26 mol%.

[00156] A person skilled in the art would understand that the amount of the second monomer occupies a broader range than the other monomers as mechanical strength and adhesivity are the critical factors in the present invention.

Third monomer: NSPP-binding monomer

[00157] As discussed above, the polymer used in the present invention can optionally be formed by combining the polymer with an NSPP. In order to effectively combine the polymer with the NSPP, preferably monomers or units that have a crosslinking ability are included in the polymer.

[00158] This crosslinking ability means that the polymers are able to bind to NSPPs and, by doing so, crosslink the NSPP to form polymer containing the NSPP. Alternatively, via a similar mechanism, the NSPPs act as the crosslinker, thereby crosslinking the polymer to form a polymer.

[00159] In order to produce a polymer that is capable of binding to NSPPs, a person skilled in the art will understand that monomers capable of binding to an NSPP need to be present in the polymers of the present invention in proportions that are sufficient to crosslink with an NSPP, such that a polymer can be formed in the presence of water. Generally, the proportion of “crosslinking” monomers in the polymer is at about 15:1, about 10:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, about 1:15 of crosslinking monomer:water binding monomer.

[00160] Monomers that are capable of binding to NSPPs generally have either electrophilic or nucleophilic functional groups, such that a nucleophilic functional group on, for example, an NSPP may react with an electrophilic functional group on the monomer, to form a covalent bond.

[00161] Therefore, for example, if an NSPP has nucleophilic functional groups such as amines, the polymer may have electrophilic functional groups such as N-hydroxysuccinimides (NHS). Other electrophilic functional groups that are suitable for use in the present invention are N-hydroxysulfosuccinimide (SNHS) and N-hydroxyethoxylated succinimide (ENHS). An example of a monomer of this type is N-acryloxysuccinimide (NAS). On the other hand, if an

NSPP has electrophilic functional groups, then the polymer may have nucleophilic functional groups such as amines or thiols.

[00162] Preferably, the polymer comprises the third monomer in an amount of up to 15 mol%. In various embodiments, the third monomer may be present in about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. In various embodiments, the third monomer may be present from about 0 to about 1, about 1 to about 15, about 2 to about 14, about 3 to about 13, about 4 to about 12, about 5 to about 11, about 6 to about 10, about 7 to about 9, or about 8 mol%.

[00163] A skilled person would understand that the polymer may be formed from hydrophobic compositions, therefore the third monomer is optional in the polymer.

Fourth monomer: Phase-transition monomer

[00164] In another embodiment of the present invention, the polymer may further include a fourth monomer that is capable of imparting phase transition characteristics to the polymer, thereby ensuring post-administration stability of the polymer. Further, these phase-transition characteristics allow the polymers of the present invention to form polymer, of which various properties (such as viscosity) can be varied by altering factors such as pH and temperature. The polymer are designed such that the lower critical solution temperature (LCST) is below body temperature. Various thermo-responsive and injectable polymers including poly(ethylene oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide) (PNIPAAm) copolymers are suitable for use in the present invention.

[00165] Generally, the proportion of phase-transition monomers in the polymer is at least about 3:1 molar ratio of phase-transition monomer:water binding monomer. This ratio can increase to, for example, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, about 16:1, about 17:1, about 18:1, about 19:1, about 20:1, about 25:1, about 30:1, about 35:1, about 40:1, about 45:1, about 50:1, about 55:1, about 60:1, about 65:1, about 70:1 molar ratio, about 75:1, about 80:1 and about 85:1 of phase-transition monomer:water binding monomer.

[00166] Preferably, the polymer comprises the fourth monomer in an amount which makes up the remainder to 100% of the polymer composition. In an embodiment, the mol% of the fourth monomer can be up to about 85%, preferably, about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or 85 mol%.

Other polymer properties

[00167] It will be understood by a person skilled in the art that, by combining different types of monomers, polymers can be produced that have a range of different properties. In addition, by incorporating particular monomers or functional groups into a pre-existing polymer, the properties of the polymer can be modified. For example, co-polymerisation of HEMA monomers with other monomers (such as methyl methacrylate) can be used to modify properties such as swelling and mechanical properties. Monomers may also be reacted with other compounds to form macromonomers (defined above) that are then included in the polymers of the present invention. For example, HEMA can be reacted with lactide to form a HEMA-poly-lactic acid polymer (PLA/HEMA), which itself can be used as a monomer in the polymers of the present invention. In addition, the monomers themselves may be combinations of monomer units, which are then incorporated into the polymer. An example of this type of monomer is oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA), which is a hydrophilic monomer composed of two hydrophilic monomers: ethylene glycol and methacrylate.

[00168] The preferred polymers of the present invention may be further modified with one or more moieties and/or functional groups. Any moiety or functional group can be used in accordance with the present invention. In some embodiments, polymers may be modified with polyethylene glycol (PEG), with a carbohydrate, and/or with acyclic polyacetals derived from polysaccharides. In addition, as discussed above, hydrophilic groups can be incorporated into monomers (and therefore polymers) to increase the water-binding capacity of the polymer.

[00169] In terms of sequence, copolymers may be block copolymers, graft copolymers, random copolymers, blends, mixtures, and/or adducts of any of the foregoing and other polymers. Typically, polymers in accordance with the present invention are organic polymers. Preferably, the polymers of the present invention are biocompatible. In some embodiments, the polymers are biodegradable. In other embodiments, the polymers are both biocompatible and biodegradable.

[00170] The preferred polymers of the present invention may also include other monomers in their structure. For example, the monomers may be polymers such as poly(vinyl alcohol) (PVA), polyesters, acrylic polymers and ionic polymers, or monomers of these.

[00171] If it is desired that the polymer be biodegradable or absorbable, one or more monomers having biodegradable linkages may be used. In the alternative, or in addition, the monomers may be chosen such that the product of the reaction between them results in a biodegradable linkage. For each approach, monomers and/or linkages may be chosen such that

the resulting biodegradable polymer will degrade or be absorbed in a desired period of time, e.g., from about 6 h to about 6 months. Preferably, the monomers and/or linkages are selected such that, when the polymer degrades under physiological conditions, the resulting products are non-toxic.

[00172] The biodegradable linkage may be chemically or enzymatically hydrolysable or absorbable. Illustrative chemically-hydrolysable biodegradable linkages include polymers, copolymers and oligomers of glycolide, lactide, caprolactone, dioxanone, and trimethylene carbonate. Illustrative enzymatically-hydrolysable biodegradable linkages include peptidic linkages cleavable by metalloproteinases and collagenases. Additional illustrative biodegradable linkages include polymers and copolymers of poly(hydroxy acid)s, poly(orthocarbonate)s, poly(anhydride)s, poly(lactone)s, poly(aminoacid)s, poly(carbonate)s, and poly(phosphonate)s.

[00173] The chemical hydrolysis of lactide in the invention results in the increase of lower critical solution temperature (LCST) of the polymer (by decreasing the overall hydrophobicity of the polymer) and thus its bioresorptive capacity.

Preferred polymers

[00174] The polymer preferably comprises the first monomer in an amount of from about 1 to about 15 mol%. In various embodiments, the first monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. Preferably, the first monomer is OEGMA.

[00175] The polymer preferably comprises the second monomer in an amount of from about 5 to about 50 mol%. In various embodiments, the second monomer may be present in about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 mol%. Preferably, the second monomer is PLA/HEMA.

[00176] The polymer preferably comprises the third monomer in an amount of up to 15 mol%. In various embodiments, the third monomer may be present in about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mol%. Preferably, the third monomer is NAS.

[00177] The polymer preferably comprises the fourth monomer in an amount which

makes up the remainder to 100% of the polymer composition, for example, from about 50 and about 85 mol%. In an embodiment, the mol% of the fourth monomer can be up to about 85%, preferably, about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or 85 mol%. Preferably, the fourth monomer is NIPAAm.

[00178] The percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

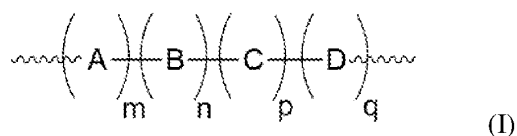
[00179] In one embodiment, the polymer preferably comprises: the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of up to 15 mol%; and the fourth monomer in an amount of up to about 85 mol%.

[00180] Preferably, the first monomer is OEGMA, the second monomer is PLA/HEMA, the third monomer is NAS and the fourth monomer is NIPAAm.

[00181] In another embodiment, the polymer preferably comprises: the first monomer in an amount of about 7 mol%; the second monomer in an amount of about 30 mol%; the third monomer in an amount of about 7 mol%; and the fourth monomer in an amount of about 53 mol%.

[00182] Preferably, the first monomer is OEGMA, the second monomer is PLA/HEMA, the third monomer is NAS and the fourth monomer is NIPAAm.

[00183] In one embodiment, the polymer of the present invention is a polymer of Formula (I):



[00184] wherein

[00185] A is the first monomer (a water-binding monomer), for example, OEGMA;

[00186] B is the second monomer (a monomer that is capable of imparting mechanical properties to a polymer), for example, PLA/HEMA;

[00187] C is the third monomer (a monomer that has a functional group for binding to an NSPP), for example, NAS; and

[00188] D is the fourth monomer (a monomer that is capable of imparting phase transition characteristics to the polymer), for example, NIPAAm.

[00189] In various embodiments, m is an integer from 1 to 20; n is an integer from 1 to

20; p is an integer from 0 to 20; and q is an integer from 1 to 20.

[00190] An exemplary polymer of the present invention is represented by Formula (I), as shown above, wherein A is the water-binding monomer OEGMA, B is the strengthening monomer PLA/HEMA, C is the crosslinker NAS, D is the phase transition monomer NIPAAm, and m, n and p, q, x and y are as defined above.

[00191] A person skilled in the art will be aware that the monomers A, B, C and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.

[00192] It has also been discovered that some monomers, such as PLA/HEMA, polyesters such as poly(lactic acid), poly(caprolactone), poly(glycolide), and their random copolymers (*e.g.*, poly(glycolide-co-lactide) and poly(glycolide-co-caprolactone) and other biodegradable and biocompatible polymers, can elevate the LCST of the preferred polymer used in the present invention during degradation of biodegradable segments (*e.g.*, PLA) *in vivo*, leading to bioresorption of the polymer. This provides the additional advantage that the polymers used in the present invention may be designed so as to be biodegradable *in vivo*.

[00193] The overall size of the preferred polymer used in the present invention may differ, depending on factors such as the types of monomers that are incorporated into the polymer, the type of NSPP that is sought to be used to form the polymer, and the conditions under which the protein is to be coupled to the polymer. However, in general, the preferred polymer used in the present invention may be a molecule of about 1 to about 100 kDa, about 5 to about 60 kDa, or about 30 kDa. In various embodiments, the polymer of the present invention may be a molecule of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or about 100 kDa.

PNPHO

[00194] A preferred polymer of the present invention is Poly(NIPAAm-*co*-NAS-*co*-(PLA/HEMA)-*co*-OEGMA), *i.e.*, “PNPHO”, *e.g.*, Formula (I). The polymer PNPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, NAS in an amount of up to 15 mol%, and NIPAAm in an amount which makes up the remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%.

[00195] The percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

[00196] In one embodiment, preferably the polymer comprises:

[00197] OEGMA in an amount of from about 3 to about 8 mol% (for example from about 4 to about 6 mol%);

[00198] HEMA-PLA in an amount of from about 5 to about 9 mol% (for example from about 6 to about 8 mol%);

[00199] NAS in an amount of at least about 7 mol%; and

[00200] NIPAAm in an amount of up to about 85 mol% (for example up to about 81 mol%).

[00201] In another embodiment, the polymer comprises:

[00202] OEGMA in an amount of about 5 mol%;

[00203] HEMA-PLA in an amount of about 7 mol%;

[00204] NAS in an amount of about 7 mol%; and

[00205] NIPAAm in an amount of about 81 mol%.

[00206] A preferred form of the polymer PNPHO for use in the present application is a polymer of Formula (I), as drawn above.

[00207] Based on Formula I, defined previously:

[00208] A is oligo (ethylene) glycol monomethyl ether methacrylate OEGMA;

[00209] B is hydroxyethyl methacrylate poly(lactic acid) (HEMA-PLA);

[00210] C is N-acryloxysuccinimide (NAS); and

[00211] D is N-isopropylacrylamide (NIPAAm).

[00212] A preferred form of the polymer PNPHO for use in the present application is a polymer of Formula (I), as drawn above. In addition, x is in the range of 1-1000 and y is in the range of 1-1000 and m, n, p, and q are in the range of 1-20.

[00213] A person skilled in the art will be aware that the monomers A, B, C and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.

PPHO

[00214] Another preferred polymer of the present invention is Poly(NIPAAm-co-(PLA/HEMA)-co-OEGMA), *i.e.*, "PPHO", *e.g.*, Formula (II). The polymer PPHO preferably comprises OEGMA in an amount of from about 1 and about 15 mol%, PLA/HEMA in an amount of from about 5 and about 50 mol%, and NIPAAm in an amount which makes up the

remainder to 100% of the polymer composition, for example, from about 50 to about 85 mol%. In preferred embodiments, PPHO comprises OEGMA in about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or about 15 mol% and/or PLA/HEMA in about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or about 50 mol% and/or NIPAAm in an amount of about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or about 85 mol%.

[00215] The percentages recited herein relate to the composition of the final polymer and not the feed amounts utilised when forming the polymer.

[00216] A preferred form of the polymer PPHO for use in the present application is a polymer of Formula (II), as drawn below. In addition, x is in the range of 1-1000 and y is in the range of 1-1000 and m, n, and q are in the range of 1-20.

[00217] A person skilled in the art will be aware that the monomers A, B, and D may be present in the polymer in any order, provided that the required water-binding, strengthening and/or cross-linking capabilities are achieved.

Synthesis of polymers

[00218] A person skilled in the art will be aware of suitable methods of synthesising the preferred polymers used in the present invention. These include methods such as ring-opening polymerisation, addition polymerisation (including free radical polymerisation) and condensation polymerisation.

[00219] The formation of the preferred polymers, PNPHO and PPHO, is described in the examples below.

Excipients and biologically-active agents

[00220] Pharmaceutically-acceptable excipients may be included in the compositions and/or polymer of the present invention, and include any and all solvents, dispersion media, inert diluents, or other liquid vehicles, dispersion or suspension aids, granulating agents, surface active agents, disintegrating agents, isotonic agents, thickening or emulsifying agents, preservatives, binding agents, lubricants, buffering agents, oils, and the like, as suited to the particular dosage form desired. Remington (Gennaro, A. R., Remington: The Science and Practice of Pharmacy, 21st Ed (2006) Lippincott Williams & Wilkins) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient is incompatible with a

substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[00221] Excipients such as colouring agents, coating agents, sweetening, flavouring, and perfuming agents can be present in the composition, according to the judgment of the formulator.

[00222] Biologically active agents or drug compounds that may be added to the composition and/or polymer of the present invention include proteins, glycosaminoglycans, carbohydrates, nucleic acids and inorganic and organic biologically active compounds, such as enzymes, antibiotics, anti-neoplastic agents, local anaesthetics, hormones, angiogenic agents, anti-angiogenic agents, growth factors (*e.g.*, insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF) and transforming growth factor- β (TGF β)), antibodies, neurotransmitters, psychoactive drugs, anticancer drugs, chemotherapeutic drugs, drugs affecting reproductive organs, genes, and oligonucleotides.

[00223] A composition containing components such excipients and/or biologically active agents can be produced by combining a polymer of the present invention with an NSPP, combining this with one or more other components and then freeze-drying the resulting composition. This leads to a ready to use polymer.

[00224] The amount of polymer, NSPP and biologically active agent present in the composition will necessarily depend upon the particular drug and the condition to be treated. A person skilled in the art will be aware of appropriate agents and amounts to use to treat the condition.

Compositions for forming hydrogels

[00225] The present invention also relates to a preferred composition useful for forming a hydrogel for use in the invention.

[00226] The composition of the present invention comprises a polymer and an NSPP, the polymer comprising:

[00227] a first water-binding monomer; and

[00228] a second monomer that imparts mechanical properties;

[00229] optionally, a third monomer that is an NSPP-binding monomer, comprising a functional group that is capable of binding to the NSPP;

[00230] a fourth monomer capable of imparting phase transition characteristics to the hydrogel;

[00231] wherein the natural or synthetic peptide or protein (NSPP) is Thymosin beta-4 or a functional homolog thereof;

[00232] and wherein the binding of the NSPP to the second monomer crosslinks the polymer, thereby enabling formation of a hydrogel when the composition is contacted with water.

[00233] The term “composition”, as used herein, refers to a solid or liquid composition containing the components mentioned above. In some embodiments, other components such as pharmaceutically-acceptable excipients and biologically active agents (*e.g.*, drugs, vitamins and minerals), to assist in repair and/or re-generation of the target bone tissue, and/or to provide a method of achieving targeted delivery of biologically active compounds, may also be included in the preferred compositions used in the present invention.

[00234] In general, the amount of polymer in the composition used in the present invention is an amount that allows for the formation of hydrogels.

[00235] In some embodiments, the amount of polymer in the composition ranges: from about 1% w/w to about 90% w/w, from about 2% w/w to about 80% w/w, from about 4% w/w to about 70% w/w, from about 5% w/w from about 60% w/w, from about 5% w/w to about 50% w/w, from about 6% w/w to about 40% w/w, from about 7% w/w to about 30% w/w or from about 8% w/w to about 20% w/w.

[00236] In some embodiments, the amount of polymer is: about 1% w/w, about 2% w/w, about 3% w/w, about 4% w/w, about 5% w/w, about 6% w/w, about 7% w/w, about 8% w/w, about 9% w/w, about 10% w/w, about 15% w/w, about 20% w/w, about 25% w/w, about 30% w/w, about 35% w/w, about 40% w/w, about 45% w/w, about 50% w/w, about 55% w/w, about 60% w/w, about 65% w/w, about 70% w/w, about 75% w/w, about 80% w/w or more. In some embodiments, the amount of polymer is approximately 85% w/w.

[00237] As a general rule, the solidity of the hydrogel increases with higher polymer concentrations in the composition.

[00238] In general, the amount of NSPP in the composition of the present invention is an amount that allows for the formation of hydrogels.

[00239] In some embodiments, the amount of NSPP in the composition ranges: from about 0.01% w/w to about 60% w/w, from about 1% w/w to about 50% w/w, from about 1% w/w to about 40% w/w, from about 5% w/w to about 30% w/w, from about 5% w/w to about 20% w/w, or from about 5% w/w to about 10% w/w.

[00240] In some embodiments, the percent of NSPP is about 1% w/w, about 2% w/w, about 3% w/w, about 4% w/w, about 5% w/w, about 6% w/w, about 7% w/w, about 8% w/w,

about 9% w/w, about 10% w/w, about 20% w/w, about 30% w/w, about 40% w/w, about 50% w/w, or more.

[00241] The % w/w is based on the total weight of the composition before the composition is contacted with water.

The polymer system as an enabling carrier mRNA and lipid nanoparticles (LNPs) and to enhance their structural and functional stability

[00242] Standard laboratory equipment was used throughout the investigation. Nasal epithelial cells RPMI 2650 are purchased from ATCC (Human squamous cell carcinoma; CCL-30), 1 x MEM (Minimum Essential Medium) from Gibco (Ref.11095-080, Lot. 2444918), 1 x Opti-MEM™ I Reduced Serum Medium, no phenol red from Gibco (Ref. 11058021), Lipofectamine™ MessengerMAX™ Transfection Reagent from ThermoFisher Scientific (Ref. LMRNA008), Clear Flat-Bottom Immuno Nonsterile 96-well Plates from ThermoFisher Scientific, 6.5 mm Transwell® with 5.0 µm Pore Polycarbonate Membrane Insert, Sterile, from Corning (Ref. 3421), Spectramax iD3 Multi-Mode Microplate Readers from Molecular Devices, USA, Gamma sterilised PNPHO powder, with 81 mol% NIPAAm, 7 mol% PLA/HEMA n=5, 7 mol% NAS and 5 mol% OEGMA. Gamma sterilised PNPHO polymer, dialysed against PBS with 5 kDa cut-off membranes, TriLink Biotechnologies CleanCap EGFP mRNA (Catalogue Number: L-7601), 1 x PBS (Phosphate Buffered Saline) from Sigma-Aldrich (Ref. 806552 Lot. RNBL1507), NanoDrop® 2000 Spectrophotometer, Thermo Scientific™ 96 Well Black/Clear Bottom Plate, TC Surface (Catalogue Number: 165305).

[00243] Analyses conducted with a range of equipment and devices, including Molecular Devices Spectramax iD3 Multi-Mode Microplate Readers, Bio-Rad Gel D^oCTM EZ Gel D^oCumentation System, nvitrogen™ SYBR™ Green II RNA gel stain 10,000X concentrate in DMSO* (Catalogue Number: S7568), Bioline 5 x DNA Loading Buffer Blue (Catalogue Number: BIO-37045), Bioline Agarose, General Purpose 100 g, Powder, Pure agarose, Gel Electrophoresis (Catalogue Number: BIO-41025), 10 x TAE Buffer (0.4 M Tris, 0.01 M EDTA and 0.2 M acetic acid).

TP and TL preparation and coding

[00244] PNPHO, dialysed PNPHO polymer and/or PPHO, dialysed PPHO polymer is dissolved in PBS and/or other buffered solution, including Hartsman's DMEM, DPBS or other suitable buffered solutions for mRNA formulations at 2 to 10 °C until a clear, single phase solution is achieved. The resulting solutions are coded as "TPXXX" (PNPHO) or "TLXXX"

(PPHO), whereas three digits “XXX” represent the mass concentration of the polymer in the buffered solutions. For example, TP050 represents 50 mg/mL of PNPPO polymer in PBS.

[00245] TP100 and TL100 were prepared with 1 x PBS or Opti-MEM media and left to gently mix overnight at 4 °C. The TP100 and TL100 solutions are stored at 4 °C until use. On the day of experiment the mRNA was mixed with the appropriate volumes of TP100 and/or TL100 solution and PBS or Opti-MEM to create the desired TP or TL solution concentrations.

[00246] For mRNA analysis, 10 µL of mRNA standards (1.95-4000 ng (final amount)) were loaded into a 2% (w/v) agarose gel incorporating SYBRTM Green II RNA gel stain in a ratio of 1:10000. The gel was run at 90 volts 40 minutes and imaged with the Gel D^oCTM EZ Gel D^oCumentation System using both intense band and faint band modes. The gel bands were quantified using Image J software. Quant-iTTM Ribogreen RNA Assay was performed according to manufacturer's protocol (<https://www.thermo fisher.com/document-connect/document-connect.html?url=https://assets.thermofisher .com/TFS-Assets%2FLSG%2Fmanuals%2Fmp11490.pdf>).

mRNA stability study 4 °C – integrity and content and nanoparticle potential

[00247] The mRNA stability study for RNA integrity and content was conducted using Aptar VP7, 5 mL nasal spray bottles where the PBS-mRNA or TP-mRNA formulations were stored at 4 °C in the spray bottles and sampled (actuated twice in falcon tube and centrifuged 100 x g, 5 min) once every day for 5 days. The mRNA content was analysed by using agarose gel electrophoresis method. The nanoparticle potential studies were conducted using the Zetasizer to measure in any nanoparticle were detected in the formulations.

mRNA stability study 37 °C – integrity and content

[00248] The mRNA stability study for RNA content was conducted using 10 mL glass vials where the PBS-mRNA or TP-mRNA formulations were stored at 37 °C and sampled (100 µL) immediately (day 0), after 24 h (Day 1) and after 96 h (Day 4). The liquid supernatant formed on top of the hydrogel in the TP50 formulations after incubation at 37 °C were also sampled on days 1 and 4 to determine if the mRNA was completely entrapped in the hydrogel layer or if it is dispersed throughout the gel and the liquid components. The samples were collected on the appointed times by incubating for 1 h in the fridge to allow the gel to form back into solution and sampling via direct pipetting and transferring to a VP7 Aptar pump for the aerosolisation collection described herein. Following sampling the formulations are placed

back into the glass vials at 37 °C to continue the incubation. The samples were analysed using agarose gel electrophoresis.

mRNA release study from TP or TL formulations

[00249] The sustained mRNA release was measured by placing the Transwell inserts (5 µm, polycarbonate membrane) with the PBS-mRNA, TP-mRNA or TL-mRNA formulations (100 µL) in a 24-well plate and adding 0.5 mL of saline to the basal chamber. The plate was then incubated at 37 °C. Samples of 100 µL were collected from the basal chamber at different time points and the same amount of fresh pre-warmed PBS was replaced after each sample collection. The assay was performed for up to 180 h. The collected samples were analysed via the Quant-iT™ Ribogreen RNA Assay to quantitate mRNA released from the formulations. The liquid dialysed polymer provided by the present Applicant was used for these experiments.

mRNA-TP formulations transport study through nasal epithelia

[00250] The nasal epithelia were established by growing the RPMI2650 cells in air-liquid interface culture using Transwell plates 24-well plates (5 µm, polycarbonate membrane). The PBS-mRNA, TP-mRNA or TL-mRNA formulations (10 ug mRNA in 100 µL) were deposited on top of the nasal epithelia using a micropipette, 500 µL of PBS is placed in the basal chamber. Samples of 100 µL were collected from the basal chamber at different time points and the same amount of fresh pre-warmed PBS was replaced after each sample collection. After 4 h, the apical surface was washed with 400 µL of PBS to collect the formulation remaining on top of (“ON”) the nasal epithelia. As the cellular mRNA would interfere with EGFP mRNA quantification, the “IN” sample quantitation was not attempted. mRNA quantitation was conducted with the Quant-iT™ Ribogreen RNA Assay, the liquid dialysed polymer provided by the present Applicant was used for these experiments.

mRNA functionality study with time lapse microscopy

[00251] The functionality of the EGFP mRNA was assessed by the cellular production of the green fluorescent protein (GFP). RPMI2650 basal cell model was used, the cells were cultured onto 24-well plates for 24 h. The media of the cells were changed to Opti-MEM media and incubated for 2 h at 37 °C. The media was then replaced again with the following:

- i. Control – 500 µL of fresh Opti-MEM media
- ii. mRNA alone – 500 ng of mRNA diluted to 500 µL with fresh Opti-MEM media
- iii. mRNA with Lipofectamine – 500 ng of mRNA mixed with 0.75 µL lipofectamine

diluted to 500 μ L with fresh Opti-MEM media

iv. mRNA with TP025/TL025 – 500 ng of mRNA diluted to 500 μ L of Opti-MEM media with 25 mg/mL of PNPPO/PPHO.

v. mRNA with Lipofectamine with TP025 – 500 ng of mRNA mixed with 0.75 μ L lipofectamine diluted to 500 μ L with fresh Opti-MEM media with 25 mg/mL of PNPPO.

Corresponding mixtures were obtained for PPHO polymer.

[00252] The 24-well plate was kept in a humidified chamber at 37 °C in 5% CO₂ atmosphere and 95% humidity, and observed using a Nikon Eclipse Ti microscope (Nikon, Tokyo, Japan) with Coolsnap ES2 camera. Photographs were taken every 2 h for 48 h using NIS-Elements (version 3.22.01) set to take images in both “Phase” and “Fluorescent” modes. The images were analysed using Fiji ImageJ. For this study the normal TP batch (STR03-075-2022-02-14-01) was used.

Direct exposure - mRNA functionality assay with microplate reader

[00253] The microplate reader functionality assay was developed to measure the efficacy of EGFP mRNA. The RPMI cells where the EGFP mRNA was assessed by the cellular production of the green fluorescent protein (GFP). RPMI2650 basal cell model was used, the cells were cultured onto 24-well plates for 24 h. The media of the cells were changed to Opti-MEM media and incubated for 2 h at 37 °C. The media was then replaced with the following:

i. Negative control – Lipofectamine 0.75 μ L diluted to 500 μ L with fresh Opti-MEM media

ii. Positive control – 500 ng of mRNA mixed with 0.75 μ L lipofectamine diluted to 500 μ L with fresh Opti-MEM media

iii. TP015 formulation – 500 ng of mRNA mixed with 0.75 μ L lipofectamine diluted to 500 μ L with fresh Opti-MEM with 25 mg/mL polymer. A corresponding TL/PPHO formulation was prepared.

iv. TP035 formulation – 500 ng of mRNA mixed with 0.75 μ L lipofectamine diluted to 500 μ L with fresh Opti-MEM with 32 mg/mL polymer. A corresponding TL/PPHO formulation was prepared.

[00254] After 24 h incubation at 37 °C in 5% CO₂ atmosphere. The plate was read using Spectramax iD3 microplate reader. The formulations were there removed from the wells and the wells were washed once with 500 μ L of cold PBS and read again (to remove any interference caused by the TP/TL on the fluorescent signal).

Parallel mRNA functionality assay with nanoparticle measurements

[00255] This study was designed to decipher if the TP/TL formulations with mRNA and lipofectamine can form nanoparticles and if these nanoparticles are able to preserve the efficacy of the mRNA. RPMI2650 basal cell model was used, the cells were cultured onto 24-well plates for 24 h. The media of the cells were changed to Opti-MEM media and incubated for 2 h at 37 °C. The media was then replaced with the following:

- i. Negative control – Lipofectamine 0.75 µL diluted to 500 µL with fresh Opti-MEM media
- ii. Positive control – 500 ng of mRNA mixed with 0.75 µL lipofectamine diluted to 500 µL with fresh Opti-MEM media

[00256] For the indirect exposure to the formulations, the media on the cells were replaced with fresh Opti-MEM media and Transwell inserts (5 µm, polycarbonate membrane) were placed on top of the wells carrying the following formulations:

[00257] (i) TP0 formulation – 500 ng of mRNA mixed with 0.75 µL lipofectamine diluted to 100 µL with fresh Opti-MEM. A corresponding TL/PPHO formulation was prepared.

[00258] (ii) TP015 formulation – 500 ng of mRNA mixed with 0.75 µL lipofectamine diluted to 100 µL with fresh Opti-MEM with 15 mg/mL polymer. A corresponding TL/PPHO formulation was prepared.

[00259] (iii) TP030 formulation – 500 ng of mRNA mixed with 0.75 µL lipofectamine diluted to 100 µL with fresh Opti-MEM with 30 mg/mL polymer. A corresponding TL/PPHO formulation was prepared.

[00260] A parallel 24-well plate was also set alongside this cell experiment with the exact same conditions but no cells. After 24 h incubation at 37 °C in 5% CO₂ atmosphere, the Transwell inserts were removed from all the wells and the cell plates were measure using the fluorescent plate reader. The media in the non-cell plates were analysed using the Zetasizer.

[00261] The data are presented as mean ± standard deviation (STDev) of three independent experiments. Statistical analysis was performed using Prism software version 9.4.0 (GraphPad, San Diego, USA).

Results: 5mRNA stability at 4 °C storage and aerosolising with VP7 Aptar nasal pump

[00262] The impact of PNPCHO or PPHO addition to the stability of mRNA upon storage at 2 to 10 °C was investigated, using agarose gel electrophoresis analysis of mRNA. For this analyses, 5 mg/mL, 10 mg/mL and 15 mg/mL of PNPCHO was used to form TP005, TP010 and TP015, respectively. Corresponding TL/PPHO formulations were prepared. The results showed

that there is a PNPFO concentration dependant signal observed in the gels close to the well, however it does not interfere with the mRNA signal and aerosolising with the VP7 Aptar nasal spray does not shear the mRNA.

[00263] The addition of PNPFO or PPHO at low concentrations of 5 mg/mL and 10 mg/mL leads to sheering and instability of mRNA, indicated by the smeared degradation signal in *Figure 2b*. However, further increase of PNPFO polymer enhanced the stability of mRNA as a very distinct and stable band observed for the formulation with 15 mg/mL of PNPFO (TP015). The quantity of the mRNA is also stable at close to 100% with TP015 (*Figure 2c*). These results confirmed that at a higher concentration, PNPFO polymer chain and its hydrophilic/ hydrophobic interaction within the formulation provide protective properties.

[00264] The results in

[00265] *Figure* showed that the addition of PNPFO or PPHO to the mRNA prevent the natural agglomeration of particles, resulted in the formation of nanoparticles. This was concluded as in the absence of PNPFO, particle size of mRNA in PBS solution was above 100 d.nm, whereas upon the addition of PNPFO even at low concentration, mRNA with TP005, TP010 and TP015 forms nanoparticles (≤ 100 d.nm).

[00266] The results showed that low concentration of PNPFO or PPHO may have detrimental impact on the stability of mRNA, potentially due to the presence of small molecular weight PNPFO/PPHO segments in the formulation. However, upon the increase to 15 mg/mL or higher, the protective nature of PNPFO/PPHO and its impact to form mRNA nanoparticles lead to form stable formulations. Therefore, for further investigations, 15 mg/mL of PNPFO/PPHO or higher concentrations of the polymer is used.

mRNA stability at 37 °C storage

[00267] Based on findings from the stability of mRNA with different concentration of PNPFO/PPHO polymers, the formulations are further challenged at an elevated temperature. As such, mRNA formulations with 15 mg/mL and 35 mg/mL of PNPFO and PPHO were tested and the mRNA stability was assessed, using agarose gel electrophoresis analysis. In the formulation with 15 mg/mL of PNPFO or PPHO and upon the increase of temperature to 37 °C, an opaque solution was formed, whereas a thin layer of hydrogel was formed in the formulation with 35 mg/mL of PNPFO with a supernatant phase at the top (

[00268] *Figure a*).

[00269] After reconstitution of the formulations with 1 h incubation at 4 °C, the samples become homogenous solutions as expected due to the reversibility of the polymers. There are

no observable differences in integrity of the mRNA when comparing direct pipetting of the formulation vs aerosolising (

[00270] *Figure a*). At Day 4 significant decreases in the content of mRNA was observed in the original formulation (without the addition of polymer). However, the addition of PNPFO/PPHO polymers in both TP015/TL015 and TP035/TL015 formulations preserve the integrity of mRNA. This was concluded that there was no significant difference ($p > 0.05$) between the total mRNA content from Day 0 to Day 4 (

[00271] *Figure b*). Additionally, analysis of the TP035/TL035 samples (supernatant on top of the hydrogel) showed presence of the mRNA within the solution, indicating that the hydrogel does not selectively entrap the mRNA and it is dispersed within both the supernatant and hydrogel.

Parallel mRNA functionality assay and nanoparticle analysis using TP/TL formulations

[00272] mRNA with lipofectamine (mRNA + LNP) formulations are tested and incorporated with 25 and 35 mg/mL of either dialysed PNPFO or non-dialysed PNPFO polymer (or their PPHO equivalents). The formulations with and without PNPFO were challenged and stored at 37 °C to assess the impact of the addition of PNPFO on the functionality of the incorporated mRNA. The results showed that the dialyses of PNPFO polymer resulted in separation of a polymer segment population and therefore leads to a different volume percentage population (shown in

[00273] *Figure a*).

[00274] The effect of addition of TP025 and TP035 (formed with either dialysed or non-dialysed PNPFO) on the functionality of eGFP-mRNA after 24 h of storage was compared with the +Control (fresh eGFP+LNP) formulation. The results in

[00275] *Figure b* showed that the addition of PNPFO polymer preserve the functionality of mRNA as significantly higher ($p < 0.001$) GFP expression was recorded in TP025 and TP035 configurations, formed with both dialysed and non-dialysed PNPFO compared to that of without PNPFO incorporation.

[00276] Specifically, formulations with dialysed PNPFO/PPHO polymer displayed greater expression; after 24 h of storage, there was no significant difference ($p > 0.05$) between the GFP expression from TP025 and TP035 to that from +Control (fresh eGFP-mRNA+LNP), which is the maximum mRNA efficacy. Therefore, the results from these investigations confirmed that the formulations of mRNA-Lipofectamine-TP (or -TL) can release functional nanoparticles into the media which are able to induce the maximal efficacy of the mRNA in

basal RPMI2650 cell model and preserve the functionality at elevated temperature and harsher conditions.

mRNA and LNP delivery

[00277] TP (formulated with PNPFO) and TL (PPHO) platform carrier systems as a universal platform for delivery of therapeutics were challenged by using different mRNA-based formulations. During the initial phase, GFP-mRNA was used and mixed with TP035, TP015 and TP007 (and their respective TL equivalent formulations) to systematically investigate the effect and potential of the inventive polymer platform carrier. Subsequently, different mixing methods by changing the sequence of mRNA, LNP and TP/TL incorporation were studied and the impact of TP/TL carriers on the encapsulation of mRNA within LNPs was assessed. Following this, TP050 and TP100 with eGF-mRNA/LNP (and TL equivalents) were formulated to ensure the compatibility of mRNA/LNPs with higher concentrations of polymer, expectably more suitable for long-acting injectable formulations. The configurations more useful for nasal delivery were then investigated and the compatibility of the carrier with a commercially available COVID-19 vaccine was assessed. The effect of the carrier layer on the biological activity of LNPs and mRNA formulations was then investigated to ensure that the carrier did not impede their intended function.

Preservation of mRNA within TP platform

[00278] eGFP-mRNA was used and mixed with PNPFO solution in PBS at 7 mg/mL, 15 mg/mL and 35 mg/mL to obtain TP007+, TP015+ and TP035+, respectively. Equivalent PPHO-comprising “TLXXX+” formulations were obtained. *Figure 11a* shows volume percentage graphs of the GFP-mRNA with and without different carrier configurations. The results in *Figure 11b* show that the Z-Ave (d/nm) value decreases significantly ($p < 0.001$) by the addition of the carrier system, regardless of its solid content, *i.e.*, for all three tested configurations ($p < 0.001$). Similarly, the results in *Figure 11c* show that polydispersity (Pdi) of the solutions was significantly ($p < 0.01$) decreased upon the addition of the carrier platform. These results indicate that the TP/TL platform carrier systems can homogeneously entangle mRNA and display protective properties against agglomeration of mRNA samples.

[00279] To test the impact of the addition of carrier system to mRNA-based therapeutics, different carrier configurations TP007+, TP015+ and TP035+ (and their TL equivalents) were collected and tested with agarose gel electrophoresis to assess the integrity of mRNA components (*Figure 14*). All test samples were stored at 4 °C for 5 days prior to the analyses.

The pH of all solutions remained around 7.3 for the duration of the test. After spraying, some degree of foaming in TP007+ was apparent. Gel electrophoresis of the solutions, using Sybr Green II stain has a good detection range of mRNA, however, the carrier component was also stained at bands with larger molecular weights (*Figure 14a*). Regardless, the separation in mRNA bands was effective and thus used to establish a standard curve for further quantification. The results in *Figure 14b* showed significant mRNA sheering in TP007+ samples whereas the extent of sheering in TP015+ and TP035+ was insignificant. This result indicates that the protective nature of the polymer carrier is concentration dependent. The protective nature of TP platform for mRNA is therefore attributed to the charge of the polymer and its capability to self-assemble at a concentration higher than its CMC.

[00280] To further evaluate the protective nature of TP platform, TP015 and TP035 configurations + mRNA (and their TL equivalents) were challenged by increasing the storage temperature up to 37 °C for 4 days. The integrity of the mRNA component was quantified by using gel electrophoresis and compared with original mRNA content. Samples were collected from stored solution (prior to spray) and after spraying/hydrogel formation. The results in *Figure 12* show that at time 0 and 24 hours post storage at 37 °C, no sheering was noticed in the integrity of mRNA in the control (no carrier), TP015 and TP035. However, after 4 days of storage at 37 °C, the integrity of mRNA samples with the carrier TP015 and TP035 was significantly ($p < 0.001$) higher than that in the control group. For instance, approximately 60% of the mRNA was present in the control group after 4 days challenge whereas nearly 100% was detected in both TP015 and TP035 groups. These results confirm that the TP/TL platforms have protective properties against temperature fluctuations and may therefore reduce the stringency of the required storage conditions for mRNA-based vaccines.

Incorporation of TP/TL platforms with mRNA/LNP formulations

[00281] To evaluate the effect of incorporating the TP/TL platforms within the manufacturing process of mRNA/LNPs, e-GPF mRNA with lipofectamine was used as a model system. The quantification of mRNA was achieved by developing a GPC-MS (gel permeation chromatography) method and establishing a standard curve with $R^2 = 0.99$ (*Figure 13a*). This method was used to assess the amount of encapsulated mRNA within LNP versus the leaked/leached amount at different time points. Following, three methods were used to incorporate the TP/TL platforms within the manufacturing process of mRNA/LNP and the results were compared with the formulation without the polymer platform (TP000/TL000; control). For the incorporation of the TP platform, a stock solution of TP100 was used and

upon mixing with eGFP-mRNA/LNP the polymer platform concentration was reduced to 35 mg/mL, hence the formulations are denoted as TP035+. These formulations include:

[00282] Standard (TP000; control): Lipofectamine solution is mixed with mRNA to form eGFP-mRNA/LNP (*Figure 13b(i)*).

[00283] Formulation (A): Mixing of eGFP-mRNA/LNP for 5 minutes, followed by the addition of TP100 to form the final mixture (*Figure 13b(ii)*).

[00284] Formulation (B): Mixing of Lipofectamine with TP100, followed by the addition of eGFP-mRNA to the mixture (*Figure 13b(iii)*).

[00285] Formulation (C): Direct addition of mRNA to TP100 solution, followed by the addition of lipofectamine to form the final mixture (*Figure 13b(iv)*).

[00286] The amount of leaked/leached mRNA from the LNPs were quantified within 2 hours post-formulation and storage in 2-8 °C in order to measure the encapsulation efficiency of the final formulations. The results showed a relatively high encapsulation efficiency in the control formulation with $81.2 \pm 1.2\%$ of the incorporated mRNA remained encapsulated within the LNPs (*Figure 13b(i)*). However, the inclusion of the TP platform by mixing the formed mRNA/LNPs with TP platform in Formulation A significantly ($p < 0.05$) increased the encapsulation efficiency to $86.4 \pm 2.1\%$ (*Figure 13b(ii)*). Following, mixing lipofectamine with TP platform prior to the inclusion of mRNA further increased the encapsulation efficiency (Formulation B) to $92.4 \pm 1.9\%$ (*Figure 13b(iii)*). Lastly, the direct addition of mRNA to TP100, followed by mixing with LNP slightly increased the encapsulation efficiency to $95.7 \pm 0.9\%$ with p value of 0.0491 (*Figure 13b(iv)*).

[00287] The results of these investigations further confirmed the compatibility of the PNPPO/PPHO polymer platforms with mRNA and LNP formulations and may increase the stability and encapsulation efficiency of the final formulations. Going forward, the method of Formulation A was used, as this allows direct incorporation of the TP carrier system with pre-made mRNA/LNP formulations, either commercially-available or under development. Therefore, despite the findings that showed higher mRNA encapsulation can be achieved with Formulations B and C, the former is preferable due to manufacturing ease.

TP/TL configurations for different routes of administration

[00288] Using the mixing method of Formulation A (above), TP035+, TP050+ and TP100+ eGFP mRNA/LNP were formulated with PNPPO polymer at concentrations of 35 mg/mL, 50 mg/mL and 100 mg/mL, respectively. The effect of the TP carrier on the particle

size and the stability of the formulations after 3, 7 and 14 days of storage at 2-8 °C were investigated using DLS and GPC analytical techniques.

[00289] The results in *Figure 15* show that at $t=0$, the particle size of the control mRNA/LNP formulation (TP000) is 88.7 ± 8.2 nm. The incorporation of TP carrier significantly increases ($p < 0.05$) the particle size in TP035+, TP050+ and TP100+ to 123.9 ± 8.0 nm, 143.1 ± 4.0 nm and 180.8 ± 9.7 nm, respectively. The results also show that mixture of TP and TL carrier systems can be formulated. As such, TP035+/TL050+ systems displayed similar particle size to that of TP035+ (no statistical difference, $p > 0.05$).

[00290] This results further confirmed that the TP and/or TL platform forms a protective layer around compounds due to its negative charge and hydrophilic/hydrophobic interaction. In relation to the Pdi measurements of the particles, all configurations showed a relatively stable Pdi within the approximate range 0.2-0.4. There was no statistically significant difference among the Pdi measurements with and without TP/TL carrier; the slight increase in Pdi that was noticed for TP100 compared to TP000 is attributed to the increase of temperature during the DLS measurement and the temperature responsive nature of the carrier system.

[00291] The protective nature of TP/TL carrier was evident after 3 days of storage at 2-8 °C in all formulations. DLS and GPC results showed that in the absence of the carrier, mRNA/LNP formulations burst, evident by the particle size <10 nm and ~0% encapsulated mRNA content in TP000. Encapsulated mRNA content in all formulations with the TP, TP/TL and TL carrier was significantly ($p < 0.001$) greater than TP000 after 3 days of storage. Encapsulated mRNA contents in TP035+, TP050+, TL050+, TP035/TL050+ and TP100 were ~70-to-80% ($p > 0.05$). The particle size measurements showed that the increase PNP/PPHO polymer content resulted in the increase of particle sizes from 71.41 ± 8.0 nm in TP035 to 117.5 ± 4.3 nm and 167.5 ± 4.6 for TP050 and TP100 respectively after 3 days of storage.

[00292] Similar findings were achieved after 7 days of incubation at 2-8 °C. In summary, GPC results confirmed that the dissociation of LNP/mRNA particles progressed in the control group no LNP/mRNA particle peak was attainable in GPC results after 7 days of storage. However, mRNA/LNPs with TP, TL or TP/TL carrier remained stable in three tested formulations.

Overtime stability of formulations

[00293] Assessing the encapsulated mRNA contents and particle size measures for

different formulations up to 14 days of storage at 2-8 °C in *Figure 16* confirmed that the presence of the carrier system increases the stability of the formulation. TP035 was the most stable formulation with more than 70% of the mRNA content remaining encapsulated 14 days post formulation and storage at 2-8 °C. More than 50% of the mRNA content was still encapsulated after 14 days, however, the particle size measurements displayed some degree of degradation at this time point.

[00294] Results from this part of the study further endorsed the finding from the previous sections and showed that the presence of TP carrier increases the stability of mRNA/LNP formulations. Additionally, the increase of PNPFO to 100 mg/mL was successfully achieved and even at such high concentration, mRNA/LNPs were more stable compared to the formulations without the carrier system. The more stable nature of formulations with higher PNPFO content, including TP050 and TP100 facilitates the formulation of long-lasting injectable configurations in future studies.

The effect of polymer platform on functionality of mRNA and LNPs

[00295] To investigate the impact of TP035 on the functionality of mRNA and LNPs, eGFP-mRNA was used and GFP expression on epithelium cells was investigated at different time points via live imaging up to 48 hours. This study was used to assess whether the addition of TP035 and its hydrophilic/hydrophobic interaction with LNPs have any detrimental impact on the functionality of mRNA and LNPs. The results in *Figure 17a(i)* and *Figure 17a(ii)* showed that eGFP without LNPs has no GFP expression properties on epithelium cells. As expected, the addition of TP035 also did not impact eGFP expression (*Figure 17a(iii)*), however, addition of lipofectamine to the eGFP-mRNA + TP035 led to expression in the cells, shown in *Figure 17a(iv)* and *Figure 17b*. The expression intensity noticed in *Figure 17b(ii)* is comparable to that achieved without TP035 carrier. The results confirmed that the addition of TP035 does not detrimentally impact the effectivity of eGFP-mRNA and the incorporated lipids.

Nasal delivery of a commercial formulation in an in vitro human model

[00296] The deposition patterns of commercial vaccine formulations a) without the carrier systems and b) with TP035+ in a simulated human nasal model are depicted in *Figure 18*. For this *in vitro* assessment, the tested formulations were sprayed into the nasal cavity model incubated at 37 °C using a standard actuator. In the control group (TP000), significant nose runoff was observed instantly following application; this was equal to ~60% of the

volume or 150 μL from 250 μL sprayed volume (*Figure 18a(i)* and *Figure 18a(ii)*). This significant volume loss and the minimal reach of the formulation to the upper regions of the nasal cavity (*Figure 18a(iii)*) are known shortcomings with nasal delivery of standard formulations, *e.g.*, without carrier systems. On the other hand, no nose runoff was noticed (*Figure 18b(i)*) in the application of TP035+ formulation. Despite the presence of the carrier system in the TP035+ formulation and its relatively high viscosity compared to the control group, aerosolisation was successful, allowing the deposition of the formulation to a wide surface area (*Figure 18b(ii)* and *Figure 18b(iii)*). White arrows show the wide coverage and rapid adhesion of the formulations with TP035+ at upper regions of the nasal cavity. The adhesive nature and instant gelation of the carrier system prevented nose and throat runoff instantly after the application of TP035+ formulation.

[00297] The deposition patterns of the formulations within 20 minutes of application for both TP000 (control) and TP035+ formulations are shown in *Figure 19a*. The results showed that due to the adhesivity of the carrier system, the volume retained at the nasal cavity was significantly higher in the TP035+ group compared to the control group (TP000) at all tested time points, *i.e.*, 0, 5, 10, 15 and 20 minutes after application (*Figure 19b*). In the TP035+ group, only minimal ($< 10 \mu\text{L}$) throat runoff was noticed within 10 minutes after administration. More importantly, the excretion of water phase during and post-gelation of TP035+ formulations led to gradual coverage of the area post application of TP035+ formulations. As such, significantly ($p < 0.001$) higher nasal cavity surface area was covered at all time points with TP035+ formulations compared to the control.

[00298] The results confirmed the high potential of the carrier system to be aerosolised with standard actuators so that the formulations can navigate through the nasal cavity. The resulting aerosol forms an adhesive hydrogel layer and thereby preventing nose and throat runoff. These findings further suggest the utility of the inventive carrier system for nasal delivery applications.

Polymer nanocarrier

[00299] Drug targeting of the brain to treat neurological disorders is exceptionally challenging due to the presence of the blood-brain-barrier (BBB). This can be explained by the presence of tight endothelial junctions in the BBB that does not allow the majority of molecules to transport from the blood circulation to the brain freely.⁽⁶⁾ To overcome this physiological barrier and allow efficient delivery of therapeutic molecules into the brain, drug administration via the intranasal route is a suitable solution. This mechanism manipulates the olfactory and

trigeminal cranial nerves to transport drugs into the brain by bypassing the BBB.⁽⁷⁾

[00300] PNPFO and PPHO are advanced biomaterials that can self-assemble and form a nanocomplex for more efficient and targeted nose-to-brain drug delivery. In this study, a nanocarrier was developed for drug loading to enhance the permeation and delivery of therapeutic agents into the brain. The NPs, composed of two biocompatible polymers, were formulated in the nasal formulations with BSA as a model drug to bypass the BBB.

Determination of the Critical Micelle Concentration (CMC)

[00301] Concentration driven micelle formation potential of PNPFO polymer was assessed (

[00302] *Figure*). To determine the critical micelle concentration (CMC) of PNPFO, serial dilutions of the PNPFO polymer from 25 mg/mL were performed. The stock solution of PNPFO was prepared by dissolving the polymer in phosphate-buffered saline (PBS) at pH 7.4. Subsequently, the stock solution and all the diluted polymeric samples were analysed using the dynamic light scattering (DLS) technique (n = 3), which is a well-suited approach established by Malvern Instrument Limited (UK) for the determination of the CMC.

[00303] Complex coacervation was employed to manufacture nanoparticles (NPs) made of the anionic PNPFO with the cationic polymer chitosan. This fabrication technique was employed to promote the stability of NPs and allow the encapsulation of charged drugs into the nanocomplex. Negatively-charged bovine serum albumin (BSA), a model therapeutic agent, was first added dropwise at a concentration of 1 mg/mL to PNPFO solution (0.5 mg/mL). Then, chitosan was used to create a nanoparticle by magnetically mixing chitosan (1 mg/mL; pre-dissolved in 1% acetic acid) and PNPFO at ratios of 1:5, 1:1 and 5:1 to understand the optimal polymer: polymer ratio for the formation of a nanocarrier. Control formulations were also prepared in the absence of BSA loading.

[00304] The physicochemical properties of BSA-loaded NPs and control NPs were investigated in terms of particle size, polydispersity index (PDI) and surface charge using a Malvern Zetasizer (Worcestershire, UK).

[00305] To quantify the encapsulation efficiency of the BSA-loaded NPs, freshly prepared PNPFO:Chitosan 1:1 samples were used to encapsulate BSA at three various concentrations, including 10, 500 and 1000 µg/mL. These formulations were filled in Amicon® filters (MWCO 30 kDa) before being centrifuged at 13.3k rpm for 15 minutes at 4 °C. The filtrate was subject to analysis by high-performance liquid chromatography (HPLC) (Shimadzu, Japan) using the Symmetry™ C18 column (4.6 mm x 5 µm x 250 mm) from

Phenomenex (California, USA).

[00306] A laser diffraction technique was used to measure the droplet size of NPs actuated from the Aptar VP7 nasal spray device. Prior to measurement, a Malvern Panalytical Spraytec (Worcestershire, UK) was equilibrated at room temperature. Three mL of optimised drug formulation (PNPHO:Chitosan 1:1 containing 1000 µg/mL BSA) was filled into the nasal pump for analysis. Once primed, the device was actuated at a 45-degree angle (n=3) in the direction of the laser beam. The blank PNPHO:Chitosan 1:1 formulation was also measured as a control.

[00307] To visualise the spray pattern of both the BSA-loaded PNPHO:Chitosan 1:1 NPs, a silicone nasal cast (Koken Co. Ltd., Bunkyo-ku, Tokyo, Japan) was utilised. Using the Aptar VP7 nasal spray pump, one spray was actuated to deposit the formulation into the nasal model. Before the experiment, the nasal cast was pre-coated evenly with a thin layer of Sar-Gel Water Indicating Paste using a clean brush. Images of the nasal cast were taken at 0, 5, 10, and 20 min.

[00308] All deposition study was performed using the optimised nanoformulation of the BSA-loaded PNPHO:Chitosan 1:1 NPs, and was pre-filled into an Aptar VP7 nasal spray device with a minimum volume of 3 mL. To determine the deposition pattern of drugs, the Aptar VP7 device was actuated three times into the in vitro nasal models including the US pharmacopoeia-approved nasal glass chamber as well as the Alberta Idealised Nasal Inlet (AINI). The flow rate was set at 15 L/min to represent nasal airflow. A QuantiPro™ BCA Assay Kit (Sigma, Australia) was used to measure the amount of BSA deposited in each representative region.

[00309] US pharmacopeia-approved nasal glass chamber. Using the FDA-approved glass chamber apparatus (Copley, UK), the nanoformulation was evaluated in terms of the percentage of drug deposition when actuated. The nasal glass chamber was attached to the next-generation impactor (NGI). After priming, drugs from the nasal spray were actuated three times into the nasal model. At the end of experiment, 25 mL of Milli-Q was used to collect the deposited drug in the glass chamber, whilst 5 mL of Milli-Q water was added to the throat and NGI stages.

[00310] Alberta Idealised Nasal Inlet (AINI) for Drug Deposition. The deposition pattern of the BSA model drug was evaluated using an NGI equipped with an AINI. The Aptar VP7 device was actuated three times into the AINI. To quantify the amount of deposited BSA in each nasal cavity assembly and NGI stages, 5 mL of Milli-Q water was used for rinsing, except in the nasopharynx of the AINI where 10 mL of Milli-Q water was required.

Reconstitution and viability of freeze-dried mRNA/LNP/TP

[00311] The following embodiment tests the viability of the inventive mRNA/LNP/polymer carrier system when freeze dried, stored and reconstituted.

[00312] A formulation with eGFP mRNA/lipofectamine (denoted as TP000) was prepared, as was an equivalent formulation having 25 mg/mL of PNPHO polymer (denoted as TP025+). Five control samples (TP000) and ten TP025+ samples, each of 200 μ L volume were prepared. All samples were frozen at -20 °C and then freeze dried at -20 °C over 36 hours. After completion of the freeze-drying process, the formed pellets were collected in their original containers (Eppendorf tubes). The samples without the carrier (TP000) and with the carrier (TP025+) were reconstituted with MilliQ water and the encapsulated mRNA content after freeze drying and reconstitution was compared with the original formulation (freshly made; no freeze-drying process).

[00313] Using the developed and previously outlined HPLC method, the mRNA encapsulation was quantified and presented in *Figure 20*. The results showed that despite the presence of the carrier system, the freeze-dried pellets can be stored and reconstituted without impacting the overall encapsulation efficiency of the formulations; there were no statistical differences between the original formulation with the carrier (TP025+) and the freeze dried formulation (TP025+FD).

[00314] Studies also confirmed that the freeze-drying process did not impact the particle size population and distributions in formulations; no statistical differences between the original formulation with the carrier (TP025+) and the freeze-dried formulation (TP025+FD). The dried formulations can be stored in ambient conditions and reconstituted prior to application. This approach, dry storage and *in situ* reconstitution may engender significant benefits for long term storage and shipment of the products.

Results and analysis*Determination of the CMC*

[00315] CMC is a crucial parameter at which polymers self-assemble to form micelles for drug encapsulation. In this study, the CMC of PNPHO polymer was reached at 0.5 mg/mL due to the initiation of self-aggregation of monomeric polymer. This was evident by the low PDI (< 0.5) at PNPHO concentrations of 0.5 mg/mL or above (

[00316] *Figure*). A PDI of < 0.5 also suggested that micelles were formed with uniform particle size distribution. Besides, particles with stable size and small hydrodynamic diameters

of < 250 nm were noted when the CMC of PNPFO was reached. For the other lower concentrations (below 0.5 mg/mL), the existence of unassociated monomer contributed to poor signal-to-noise detection in DLS. As shown in

[00317] *Figure* , samples with PNPFO concentration of <0.5 mg/mL present either a high PDI (>0.5) or large particle size (>1000 nm), suggesting that the self-assembly process of NP had not commenced.

[00318] From the initial CMC data, one may expect that the PNPFO concentrations at 0.5 mg/mL or above were suitable for drug encapsulation due to the commencement of the self-assembly process for NPs formation. From the surface charge measurement, it was discovered that PNPFO polymer was negatively charged at all tested concentrations. Such surface property allowed subsequent chemical interaction with positively charged polymers for the formation of a drug-encapsulated nanocomplex via electrostatic interaction. In addition, the inclusion of a cationic polymer in the nasal formulation further improved the stability and integrity of the nanostructure for efficient loading of anionic drug molecules.

Dynamic light scattering analysis

[00319] Chitosan was chosen as a model polymer with a positively charged properties for the formulation because of its good tolerability and permeation-enhancing effects. These properties are all important for development of an intranasal drug formulation. As compared to blank NPs, the encapsulation of BSA as a model drug in PNPFO:Chitoan (1:1) formulation significantly reduced the particle size ($p < 0.05$) from 313.97 ± 43.71 nm to 112.41 ± 15.27 nm (

[00320] *Figure*). This indicated that BSA is a compatible model drug with stabilising effect on the nanocomplex.

[00321] For other NP formulations made of PNPFO:Chitosan at ratios of 5:1 and 1:5, there were no significant effects on the particle size ($p > 0.05$). Therefore, only PNPFO:Chitosan at a ratio of 1:1 was selected for further physicochemical and aerodynamic testing.

[00322] When NPs were prepared at different ratios of PNPFO:Chitosan, it was found that the polymers at 1:1 ratio offered the most promising physicochemical properties. This was not seen in other formulations with polymer ratios at 5:1 and 1:5, by which a high PDI indicated the formation of highly dispersed particles.

[00323] As expected, the PNPFO:Chitosan at a 1:1 ratio proved to be the best formulation due to a significant decrease in particle size. This finding indicated that the addition of BSA produced NPs with more compact nanostructure, possibly via electrostatic

interaction.⁽¹²⁾ For NPs prepared at polymer ratios of 1:5 and 5:1 (PNPHO/Chitosan), no significant change in particle size was reported during BSA encapsulation into the complexes, indicating that the stabilising effect of BSA was only present under the optimal ratio of polymers in the formulation.

[00324] As promising properties was observed in NPs with PNPHO:Chitosan at 1:1 ratio, BSA was selected for drug encapsulation into the nanocomplex. BSA is a common model protein due to its relatively low cost, availability, and compatible charge. Its potential in formation of nanocomplex with oppositely charged polymers such as chitosan has been described in previous literature.^{(10),(11)} After BSA was loaded into the nanocomplex made of various PNPHO:Chitosan ratios, the hydrodynamic diameter of the fabricated particles was measured.

Determination of encapsulation efficiency

[00325] The results in

[00326] *Table 1* show that when PNPHO:Chitosan at a ratio of 1:1 was used for BSA encapsulation (10, 500 or 1000 µg/mL), all formulations had encapsulation efficiencies of 98-100%. In addition, the PDI of all the NPs had no significant difference ($p > 0.05$).

Table 1. Encapsulation efficiency and PDI of PNPHO:Chitsoan 1:1 NPs loaded with different concentrations of BSA as a model protein

NP configuration	BSA Loaded (µg/mL)	PDI	Encapsulation efficiency
PNPHO: Chitosan (1:1)	1000	0.45 ± 0.06	98-100%
	500	0.46 ± 0.05	98-100%
	10	0.45 ± 0.14	98-100%

Laser diffraction measurement

[00327] The results in

[00328] *Table 2* show the droplet size feature of nasal formulations. No significant difference in droplet size ($p > 0.05$) was seen with the Dv10 of the blank NPs and BSA-NPs. In terms of the Dv50, or median size value, BSA-loaded PNPHO:Chitosan 1:1 formulation presented a droplet size of 51.84 ± 1.40 µm. In contrast, for the control formulation, a significantly smaller median droplet size of 36.76 ± 1.64 µm was recorded ($p < 0.05$). This droplet size pattern was also observed with the Dv90 characterisation, by which a significantly larger droplet size was noted in the BSA-loaded formulation (140.84 ± 8.78 µm) than in the

blank nanoformulation (67.31 ± 3.09) ($p < 0.05$), suggesting the presence of protein drugs in the nanoformulation can influence the droplet nature of nasal sprays.

[00329] The encapsulation efficiency of the PNPHO:Chitosan 1:1 formulation was shown to be consistently high with at least 98% of BSA encapsulated. The capability of drug encapsulation into the nanocarrier was independent of the initial concentrations of BSA addition within the range of 100 and 1000 $\mu\text{g/mL}$. This can be explained by the saturation theory, by which protein encapsulation using electrostatic interaction only decreases the encapsulation efficiency once the maximum protein concentration has been loaded.⁽¹³⁾ For successful intranasal drug delivery, it is important to have a nanocarrier with high encapsulation efficiency so that drugs can be effectively delivered to the desired brain region with minimal wastage. An increase in the encapsulation efficiency of NPs is directly proportional to a higher amount of drug reaching the CNS.⁽¹⁴⁾ As BSA-loaded NPs at all drug concentrations presented high encapsulation efficiencies of 98-100%, further characterisations in terms of droplet size properties and drug deposition pattern were performed using BSA (1000 $\mu\text{g/mL}$)-loaded PNPHO:Chitosan 1:1 formulation. The formulation with the highest initial drug concentration had a greater potential to meet the therapeutic dose and achieve an effective CNS effect in the brain.

Table 2. Spraytec data showing droplet spray size

Formulation	BSA	Dv10 (μm)	Dv50 (μm)*	Dv90 (μm)*
PNPHO:Chitosan (1:1)	+	20.94 ± 0.37	51.84 ± 1.40	140.84 ± 8.78
	-	20.51 ± 1.16	36.76 ± 1.00	67.31 ± 3.09

Silicone nasal cast

[00330] Over 20 min of analysis, increasing amount of drug deposition was seen in the nasal cavity for the BSA-loaded PNPHO:Chitosan 1:1 formulations. In particular, the turbinate region demonstrated a relatively significant amount of drug deposition, as indicated by the bright pink colour of the Sar-Gel (

[00331] *Figure*).

[00332] In the present study, PNPHO polymer was characterised in relation to its CMC to understand the minimum concentration required for the formation of NPs. Subsequently, PNPHO at its CMC was utilised to form a nanocomplex by adding the cationic polymer chitosan via electrostatic interaction. This manufacturing approach allows the model

therapeutic drugs with a negative surface charge to be encapsulated. The effects of polymer ratio and initial drug concentration on the physicochemical properties of NPs were determined. The obtained data indicated the potential of a nano-drug delivery platform is ready to be loaded with various CNS drugs due to its promising physicochemical features.

Deposition study of the Pfizer Comirnaty vaccine in saline and TP035 formulations

[00333] The Pfizer-BioNTech Comirnaty vaccine has been one of the most popular vaccines in the market for immunisation against COVID-19 infection. While it has proven effective it has some restriction regarding cold-chain transport and storage requirements. This series of studies was conducted to determine if this vaccine can be used in combination with PNPHO polymer (TP carrier system) or PPHO polymer (TL carrier system) for its application for nasal delivery. The nasal cast deposition study, using the BIVAX nasal spray device (

[00334] *Figure*) demonstrates that the TP035 formulation can be deposited region specifically (upper olfactory region) and that deposition is sustained for up to 20 min post deposition. No visible dripping was observed for that period. While the saline formulation was deposited across a larger region of the nasal cavity and spread out further to cover most of the nasal cavity by 20 min. Visible dripping to the back on the throat was observed. Comparable results were observed for the TL035 formulation.

[00335] Retention/adhesion of the active/s throughout the upper respiratory region is strongly suggestive of the hydrogel nature of the polymer working as a vehicle to “glue” the active/s at the site, preventing throat/nose dripping and in turn maximising the bioavailability of the active/s.

Industrial Applicability

[00336] It will be appreciated that the present invention finds ready applicability in the biomedical and vaccine fields. The biocompatible PNPHO or PPHO polymers can be seen to provide an effective intranasal delivery vehicle for existing mRNA/LNP COVID-19 vaccines such as the popular Pfizer-BioNTech vaccine.

[00337] The key findings of the present investigation were:

- i. At any concentration above 0.5 mg/mL, PNPHO or PPHO polymers can form a micelle like configurations (at ambient condition, *i.e.*, not in its hydrogel status).
- ii. Such micelles can be stabilised ionically. One of skill in the art will note that the examples in relation to BSA encapsulation efficiency is not critical; the BSA example is mostly to show the mechanism of action of the system in relation to its ionic interactions.

- iii. The protective nature of PNPFO/PPHO (potentially justifiable based on its CMC) is concentration dependant. The results showed that at low PNPFO concentration (5 mg/mL and 10 mg/mL), the addition of polymer resulted in sheering of mRNA and thus has detrimental impact on mRNA integrity. However, at 15 mg/mL or higher, the addition of PNPFO/PPHO protects mRNA against sheering.
- iv. Such protective characteristics were further challenged at 37 °C. The results showed that 35 mg/mL of PNPFO/PPHO, the addition of the polymer preserve the integrity of mRNA for at least 4 days at the tested harsher conditions.
- v. Testing the functionality of mRNA+LNP with and without the addition of PNPFO showed that the addition of PNPFO at the concentration of 25 mg/mL and 35 mg/mL preserves the functionality of mRNA+LNP for at least 24 hours as significantly higher eGFP expression was noticed in the formulations with PNPFO addition compared to the control (stored mRNA+LNP). The use of dialysed PNPFO (and therefore separation of fragmented/ PNPFO polymer) resulted in the formulations that can release functional nanoparticles into the media to induce the maximal efficacy.
- vi. The results showed that the PNPFO/PPHO carrier systems are compatible with certain commercial COVID-19 vaccines, which may involve a cocktail of mRNA and LNPs to act as a physical vehicle system for intranasal delivery.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A polymer for forming a solution and/or hydrogel to stabilise one or more
pharmaceutically active agents prior to, during or post-administration, the polymer
5 comprising:
a first monomer for binding water;
a second monomer for imparting mechanical properties to the scaffold;
optionally, a third monomer for binding to a natural or synthetic peptide or
protein (NSPP); and
10 a fourth monomer for imparting phase-transition behaviour.
2. A polymer according to claim 1, wherein the administration is by way of
intravascular, intramuscular, subcutaneous, inhalable respiratory, oral inhaled or
intranasal administration.
- 15 3. A polymer according to claim 2, wherein the administration is intranasal.
4. A polymer according to any one of the preceding claims, wherein the first monomer
is one or more polyethers, selected from: polyethylene glycol (PEG), oligo(ethylene
20 glycol) (OEG), polyethylene oxide (PEO), polyethylene oxide-co-propylene oxide
(PPO), co-polyethylene oxide block or random copolymers thereof.
5. A polymer according to claim 4, wherein the first monomer is oligo (ethylene) glycol
monomethyl ether methacrylate (OEGMA).
- 25 6. A polymer according to any one of the preceding claims, wherein the second
monomer is a methacrylate, or a random co-polymer comprising a methacrylate,
selected from: hydroxyethyl methacrylate (HEMA), a hydroxyethyl methacrylate
poly(lactic acid) copolymer (PLA/HEMA), poly(lactic acid), poly(caprolactone),
30 poly(glycolide), poly(glycolide-colactide) or poly(glycolide-co-caprolactone).
7. A polymer according to claim 6, wherein the second monomer is hydroxyethyl
methacrylate poly(lactic acid) (PLA/HEMA).

8. A polymer according to any one of the preceding claims, wherein the third monomer has electrophilic functional groups for binding to the NSPP.
9. A polymer according to claim 8, wherein the third monomer is selected from: N-hydroxysulfosuccinimide (SNHS), N-hydroxyethoxylated succinimide (ENHS), and N-acryloxysuccinimide (NAS).
10. A polymer according to any claim 9, wherein the third monomer is N-acryloxysuccinimide (NAS).
11. A polymer according to any one of the preceding claims, wherein the fourth monomer has a lower critical solution temperature (LCST) less than about 37 °C.
12. A polymer according to claim 11, wherein the fourth monomer is selected from: poly(ethylene oxide)/poly(propylene oxide) and poly(N-isopropylacrylamide) (PNIPAAm) homopolymers and copolymers.
13. A polymer according to claim 12, wherein the fourth monomer is (N-isopropylacrylamide) (NIPAAm).
14. A polymer according to any one of the preceding claims, wherein the polymer comprises the first monomer in an amount of from about 1 to about 15 mol%; the second monomer in an amount of from about 5 to about 50 mol%; the third monomer in an amount of from about 0 to about 15 mol%; and the fourth monomer in an amount which makes up the remainder to 100% of the polymer.
15. A polymer according to any one of the preceding claims, wherein: the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm,
- wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15 mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%.

16. A polymer according to any one of the preceding claims, wherein the one or more pharmaceutically-active agents comprise a vaccine otherwise restricted to delivery via intramuscular injection.
- 5 17. A polymer according to claim 16, wherein the vaccine otherwise restricted to delivery via intramuscular injection is a COVID-19 (SARS-CoV-2) vaccine.
18. A polymer according to claim 17, wherein the COVID-19 vaccine is the Pfizer-BioNTech (Comirnaty/Tozinameran) vaccine.
- 10 19. A polymer according to any one of the preceding claims, wherein the polymer is present in a concentration greater than or equal to about 15 mg/mL.
20. A polymer according to claim 19, wherein the polymer is present in a concentration
15 between about 25 and about 35 mg/mL.
21. A polymer according to claim 20, wherein the polymer is present in a concentration of about 35 mg/mL.
- 20 22. A polymer according to any one of the preceding claims, wherein
the first monomer is OEGMA; the second monomer is PLA/HEMA; the third monomer is NAS; and the fourth monomer is NIPAAm;
wherein the polymer comprises: OEGMA in an amount of from about 1 to about 15 mol%; PLA/HEMA in an amount of from 5 to about 50 mol%; NAS in an
25 amount of from 0 to about 15 mol%; and NIPAAm in an amount of up to about 85 mol%;
wherein the vaccine is an mRNA vaccine, preferably the Pfizer-BioNTech (Comirnaty/Tozinameran) COVID-19 vaccine;
wherein the polymer is present in a concentration of about 35 mg/mL; and
30 wherein the polymer preserves the integrity of the mRNA at 37 °C for up to 4 days.
23. A method for the delivery of one or more pharmaceutically active agents, the method comprising administration to a subject in need thereof an effective concentration of

the one or more pharmaceutically active agents dispersed within a polymer as defined according to any one of claims 1 to 22.

5 24. A method according to claim 23, wherein the administration is performed at ambient temperature, with the polymer transitioning to its hydrogel form at higher (*e.g.*, body) temperatures.

25. A method according to claim 23 or claim 24, wherein the administration is performed intranasally, by intranasal spray or deposition.

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26. A method according to any one of claims 23 to 25, wherein the one or more pharmaceutically active ingredients may be dispersed within the polymer *in situ*, or pre-formulated and stored under conditions supporting viability of the active/s.

15 27. A method according to claim 26, wherein the conditions supporting viability of the active/s comprise freeze-drying the formulation prior to reconstitution of it.

28. Use of a polymer as defined according to any one of claims 1 to 22 in the manufacture of a medicament for the delivery of one or more pharmaceutically active agents.

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29. Use according to claim 28, wherein the administration is performed at ambient temperature, with the polymer transitioning to its hydrogel form at higher (*e.g.*, body) temperatures.

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30. Use according to claim 28 or claim 29, wherein the administration is performed via intranasal spray or deposition.

31. Use according to any one of claims 28 to 30, wherein the one or more pharmaceutically active ingredients may be dispersed within the polymer *in situ*, or pre-formulated and stored under conditions supporting viability of the active/s.

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32. Use according to claim 31, wherein the conditions supporting viability of the active/s comprise freeze-drying the formulation prior to reconstitution of it.

33. A kit for enabling delivery of one or more pharmaceutically active agents, the kit comprising a polymer as defined according to any one of claim 1 to 22; an effective concentration of the one or more pharmaceutically active agents stored under appropriate conditions; and optionally instructions for adding an effective concentration of the polymer to the one or more pharmaceutically active agents.
34. A kit according to claim 33, wherein the polymer and the one or more pharmaceutically active agents in pre-mixed form.