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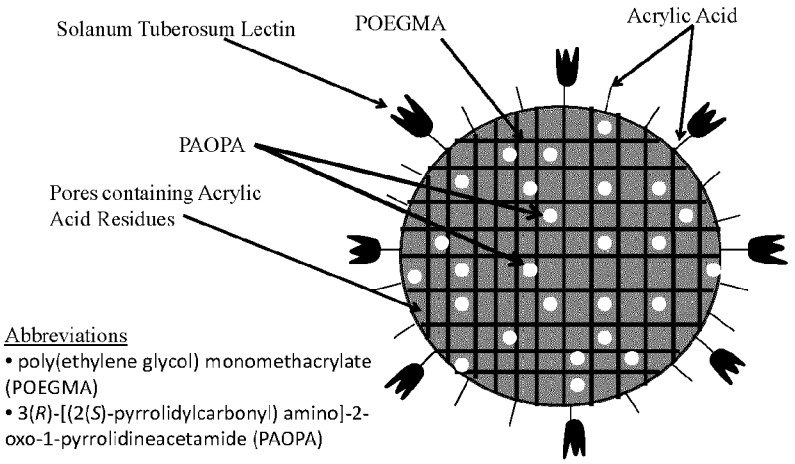
[Continued on next page]

(54) Title: MICROGEL COMPOSITIONS FOR DELIVERY OF SUBSTANCES TO THE BRAIN

Figure 1



(57) Abstract: The present application relates to microgel compositions suitable for delivery of substances to the brain. In particular, the present application is directed to microgel compositions comprising a chemically cross-linked microgel and a substance that one wishes lo deliver to the brain, for example for therapeutic or diagnostic purposes, wherein the substance is entrapped in and/or on the chemically cross-linked microgel. In an embodiment, the microgel is surface functionalized for delivery via intranasal administration. Methods and uses of the microgel compositions are also included in the application.

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TITLE: MICROGEL COMPOSITIONS FOR DELIVERY OF SUBSTANCES TO THE BRAIN

RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of co-pending United States provisional patent application no. 61/872,021, filed on August 30, 2013, the contents of which are incorporated herein by reference in their entirety.

FIELD

[0002] The present application relates to polymeric materials and their uses, in particular, the present invention relates to hydrogel materials and their use, for example, as drug delivery vehicles and biosensors.

BACKGROUND

[0003] Nanoparticle-based formulations have been investigated for increasing the likelihood of drugs reaching their target of interest (e.g. preventing binding of the drug to mucus), which could significantly improve the therapeutic efficacy and allow for dosage reduction due to a reduction in premature drug metabolism (Illum, 2002; Singh & Lillard, 2009). Customization of nanoparticles to discourage particle aggregation (avoiding biological clearance mechanisms) and promote disease or cell-specific localization can help address the challenges of specific types of drug transport (Dhuria, Hanson, & Frey, 2010; Sahoo & Labhasetwar, 2003; Singh & Lillard, 2009). Drug carrier development specialized for various routes of administration and drug polarity are factors for consideration when encapsulating drugs used in the treatment of a variety of disorders, including CNS disorders (e.g. Schizophrenia, Parkinson's disease, etc.) (Sahoo & Labhasetwar, 2003).

[0004] Nanoparticles have been tested in rodent models using intranasal, depot/injectable (subcutaneous, intramuscular, intraperitoneal, intravenous), and oral routes of administration to treat schizophrenia (Ereshefsky & Mascarenas, 2003; Kumar *et al.*, 2008). The route of administration can also be chosen based upon the desired speed of therapeutic action (Ereshefsky & Mascarenas, 2003). For example, intravenous injection may be desired when expedited action is needed for the control of acute severe symptomatology, whereas oral administration using slow-release capsules can be used in the long-term treatment of schizophrenic symptomatology (Ugwoke *et al.*, 1999). The intranasal route of administration has been found to be the

fastest route to the brain and results in high drug bioavailabilities due to a greater CNS uptake, which could be desirable if a rapid clinical effect is desired (Chhajed, Sangale, & Barhate, 2011; Kumar *et al.*, 2008; Luppi *et al.*, 2010; Ugwoke *et al.*, 1999).

[0005] Microgels are solvent-swollen networks visible as discrete particles ranging from 20 nm to 50 μm in size and have recently been growing in popularity due to their great potential as a method of drug delivery specifically as a result of cell or tissue targeting (de Jong & Born, 2008; Pelton, 2012). These microgels have hydrophilic water-swollen networks that present a low interfacial energy under biological conditions, reducing opsonisation (non-specific interactions with proteins), increasing their bioavailability and reducing the likelihood of an immune response (Smeets & Hoare, 2013). Highly monodisperse microgels can be made with sizes between 80-150 nm (Smeets & Hoare, 2013). 3-(4,5 Dimethylthiozol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays have shown that many classes of microgels are non-toxic to cells. Qiao et al. have shown that poly [oligo(ethylene glycol) methyl ether methacrylate] (*POEGMA*) microgels maintain high viability in MCF-7 and HeLa cells up to concentrations of 0.2 $mg \cdot mL^{-1}$ while paclitaxel-loaded microgels displayed a concentration-dependent cytotoxicity, demonstrating that these drug-loaded microgels are therapeutically effective in killing cancer cells (Qiao *et al.*, 2011). Sivakumaran et al. have demonstrated similar high cell viability in the presence of acid-functionalized poly(N-isopropylacrylamide) (*PNIPAM*) microgels (Sivakumaran, Maitland, & Hoare, 2011). In addition, these formulations have demonstrated excellent encapsulation efficiencies and loading capacities when the gel composition is tuned to match the drug chemistry (Siemoneit *et al.*, 2006). Peng *et al.*, 2012 produced pH-ionizable poly(N-isopropylacrylamide)-poly(methacrylic acid) (PNIPAM-PMAA)-POEGMA microgels for the delivery of the hydrophilic molecule, bovine serum albumin (BSA). Despite the relatively large size of BSA (molecular radius ~16 nm), loading capacities of > 300 mg BSA / mg dry microgel could be achieved (Qiao *et al.*, 2011; Sun & del Rosario, 1970). Release of BSA was significantly slower at pH = 1.2 (gastric pH) due to the more collapsed state of the microgel (and thus the slower diffusion of the macromolecule through the matrix) relative to pH 7.4, at which point the microgel is swollen.

[0006] There have been reports of hydrophilic drugs entrapped in microgels. One study reported significantly high encapsulation efficiencies of the antibiotic drug cefadroxil into PVA-grafted-acrylamide (PVA-g-AAm) microgels (82-95%), though these particles were very susceptible to pH-responsive swelling and rapid drug release at physiological pH (Rao *et al.*, 2006). Another group reported the absorption of hydrophilic fluorescent dyes in multi-responsive hyperbranched poly(ether amine) microgels (hPEA-mGel), where based upon the polymer-dye electrostatic interactions, less hydrophilic drugs were more likely to partition out of the microgels during centrifugation (ex. 90% with fluorescien) (Li, Jiang, & Yin, 2012).

[0007] There have been several studies analyzing the effects of modifying the sizes of drug-loaded microgels or microgels for the administration of a variety of drugs (Luo, Kirker, & Prestwich, 2000; Murphy *et al.*, 2007; Wu *et al.*, 2007). One study included a modified calmodulin (CaM) protein linked to a PEG-based microgels through selective sulfhydryl groups engineered on the CaM molecule (Murphy *et al.*, 2007). Another study used a sequential polymerization strategy to create hydrophobic dumbbell-shaped OEGMA/ *n*-butyl methacrylate (*n*BuMA) microgels at sizes of 30-60 nm following disulfide bridge cleavage using dithiothreitol (DTT) without any drug loading (He *et al.*, 2009). Precipitation polymerizations of $M(EO)_2MA$ and OEGMA300 or OEGMA475 have been shown to produce colloidally stable microgels between 100-400 nm size (Cai, Marquez, & Hu, 2007; He *et al.*, 2009), as achieved in this technology as well. One study found the density of cross-linking agent added had a dramatic effect on drug loading, which played a role in equilibrium swelling of microgels and therefore the size (i.e. increased encapsulation lead to increased swelling) (Rao *et al.*,2006).

[0008] 3(R)-[(2(S)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (PAOPA) is a positive allosteric modulator that selectively targets the dopamine D2 receptor and has been shown to improve agonist binding with both bovine and human dopamine D2 receptor with no observed effect on antagonist binding (Mishra *et al.*, 1990; Verma *et al.*, 2005). PAOPA is a highly polar molecule and, as such, does not have high bioavailabilities within the brain after intranasal administration (Illum, 2002). In fact many of the drugs used to treat schizophrenia or other central nervous system (CNS) disorders lack an effective

means for crossing the blood brain barrier (BBB) (Chen *et al.*, 2011). Any drug targeting a particular substrate within the brain needs to pass through the BBB through either passive or facilitated transport. The capacity of the drug to do this is dependent on the drug lipophilic character and molecular size (Dhuria, Hanson, & Frey, 2010). The drug carrier also typically needs to be much smaller than 150 nm in size to pass the blood brain barrier (Gaumet *et al.*, 2008). This size allows for passive diffusion through the tight junctions found in the membranes of the endothelial cells of the ethmoid artery (branch of the internal carotid artery) (Veiseh, Gunn & Zhang, 2010).

SUMMARY

[0009] Microgels as drug delivery nanoparticles possess many of the advantages needed to overcome BBB issues as they are easy to synthesize, monodisperse in size, offer long-term stability, shelf-life, and biocompatibility. Microgels have been identified herein as ideal candidates for the encapsulation and release of CNS drugs (particularly APDs) to their targets within the brain, which is generally at pH 7.4 as well as 37°C. As far as the Applicant is aware, no microgel formulation has previously been used for the encapsulation of CNS therapeutics let alone APDs or even been studied to determine neural bioavailability.

[0010] An additional advantage to the use of microgels for delivery of drugs across the blood brain barrier is the ease of surface conjugation to the microgel with high yields, resulting in a good bioavailability due to the interaction of the graft or functionalization with its targets (i.e. microgels are hydrated, have a high mobile structure and low non-specific adsorption to sterically block ligand-target binding). Another way to increase the likelihood of reaching the brain through the intranasal route of administration is through using *Solanum tuberosum* lectin (STL) for targeting the nasal epithelial cells, which has been shown to increase neural biodistribution of lipophilic dyes (Chen *et al.*, 2011). By functionalizing the surface of the microgel with *Solanum tuberosum* lectin (STL), the APD drug carriers bind more selectively to the N-acetylglucosamine residues highly expressed on the nasal epithelial membrane (Chen *et al.*, 2011; Lundh, Brockstedt, & Kristensson, 1989; Pastor *et al.*, 1992). This selective binding has been previously shown to lead to increased nasal epithelial cell uptake and improved bioavailability within the brain (Chen *et al.*, 2011; Vila *et al.*,

2005). Prior research has also shown that STL activity is unchanged upon heating up to 50°C and remains relatively stable over a large pH range (i.e. pH 4-10) (Chen *et al.*, 2011). Until the present application, such targeting has not yet been demonstrated using APD-loaded microgels. While antibodies such as immunoglobin, have also been immobilized onto microgels, these antibody-microgel conjugates have not been used for direct cell targeting or drug delivery (Silva *et al.*, 2006; Su *et al.*, 2008; Zhou *et al.*, 2004). Thus, the present application represents the first time that microgels have been functionalized for cell-targeting for the nasal route of administration for CNS-active compounds, such as APDs. The above properties of the lectin target, coupled with the relatively low nanoparticle surface charge (another particular advantage of microgels, which are largely sterically stabilized), inhibits particle aggregation, reduces interaction with the mucin molecules, and avoids the nasal clearance mechanisms.

[0011] The compositions of the present application demonstrate promise for reducing the drug dose necessary to produce a therapeutic effect with, for example antipsychotic drugs for the treatment of schizophrenia, using a non-invasive route of administration. As far as the Applicant is aware the microgel composition of the present application is the first to be shown to cross the BBB and use a surface STL-functionalization for route of administration specific cell-targeting (i.e. the nasal epithelial cells). In addition, this composition is the first use of the novel allosteric modulator PAOPA within a drug carrier formulation, a drug with extremely favorable properties for the treatment of APD.

[0012] Accordingly, in an embodiment, the present application includes a microgel composition comprising:

(a) a chemically cross-linked microgel having a particle size of about 20 nm to about 300 nm; and

(b) one or more substances for delivery to the brain,

wherein the substance is entrapped in and/or on the chemically cross-linked microgel.

[0013] In an embodiment of the application, the chemically cross-linked microgel is surface-functionalized with one or more molecules for cell targeting and/or internalization.

[0014] In an embodiment, the chemically cross-linked microgel is biodegradable.

[0015] In an embodiment the substance that modulates one or more biological targets in the brain is an antipsychotic drug. In a further embodiment, the antipsychotic drug is PAOPA or a PAOPA analog or derivative.

[0016] In another embodiment, the present application includes a pharmaceutical or diagnostic comprising one or more of the microgel compositions of the application and a pharmaceutically acceptable carrier, diluent and/or adjuvant.

[0017] In another embodiment, the present application includes a method of treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain comprising administering, to a subject in need thereof, an effective amount of one or more microgel compositions of the application, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain.

[0018] The present application also includes a use of one or more microgel compositions of the application for treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain. Also included is a use of one or more microgel compositions of the application for preparation of a medicament for treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain. Further included is a microgel composition of the present application for use in treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain.

[0019] In another embodiment, the present application includes a method of imaging the brain comprising administering, to a subject in need thereof, an effective amount of one or more microgel compositions of the application, wherein the one or

more microgel compositions comprise one or more substances that image one or more areas of the brain, and performing an imaging method on the subject.

[0020] The present application also includes a use of one or more microgel compositions of the application for imaging the brain, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain. Also included is a use of one or more microgel compositions of the application for preparation of a medicament for imaging the brain, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain. Further includes is a microgel composition of the present application for use in imaging the brain, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain.

[0021] Other features and advantages of the present application will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating embodiments of the application, are given by way of illustration only and the scope of the claims should not be limited by these embodiments, but should be given the broadest interpretation consistent with the description as a whole.

DRAWINGS

[0022] The embodiments of the application will now be described in greater detail with reference to the attached drawings in which:

[0023] Figure 1 is a schematic outlining the basic structure of the *Solanum tuberosum* lectin functionalized PAOPA-loaded POEGMA microgels in an exemplary embodiment of the present application.

[0024] Figure 2 shows the morphology of the exemplary microgels at 150,000x magnification (POEGMA microgels + PAOPA and STL-POEGMA microgels + PAOPA) via a transmission electron microscopy (TEM).

[0025] Figure 3 shows the *in vitro* leakage of PAOPA from exemplary microgels and STL-functionalized microgels in physiological conditions (10 mM PBS pH 7.4, $n = 3$). This demonstrates the minimal drug leakage within the time period of

transport of the microgels to the brain over 96 hours and the potential for efficient drug transport to the targeted brain tissue.

[0026] Figure 4 shows the degradation of the exemplary POEGMA microgels with the intact microgel solution prior to degradation on the left and the degraded microgel solution on the right following 12 hours of incubation in GSH.

[0027] Figure 5 shows the viability of RPMI 2650 nasal septum carcinoma cells following 24 hr exposure to either PAOPA, POEGMA or exemplary STL-POEGMA microgels and incubation at 37°C (n = 4/treatment). These results show that these exemplary microgel formulations are not toxic to the nasal epithelial cells during intranasal administration before, during or after exposure to the formulation.

[0028] Figure 6 shows the viability of SHS 5Y neuronal cells following 24 hr exposure to either PAOPA, POEGMA or exemplary STL-POEGMA microgels and incubation at 37°C (n = 4/treatment). These results show that these exemplary microgel formulations are not toxic to the neuronal cells before, during or after exposure to the formulation.

[0029] Figure 7 shows the brain tissue concentrations of rhodamine-labeled exemplary microgels following intraperitoneal (IP) injection in rats at 10 mg/kg of varying microgel sizes (n=5) in the prefrontal cortex (PFC), striatum and cerebellum. Microgel concentrations within the prefrontal cortex (PFC), striatum and cerebellum were measured to see whether the microgels could cross the BBB to transport PAOPA to its target, the dopamine D2 receptor. Transport was confirmed even for larger microgels (~250 nm), showing that the exemplary microgels were effectively transported into the brain.

[0030] Figure 8 shows the major organ tissue concentrations of rhodamine-labeled exemplary microgels following intraperitoneal (IP) injection at 10 mg/kg of varying microgel sizes (n=4) in the liver, lungs, spleen and kidneys. This was to determine whether microgels of smaller sizes had greater bioavailabilities after treatment. The smaller rhodamine-labelled exemplary microgels had far greater major organ tissue concentrations than the larger microgels.

[0031] Figure 9 shows the brain tissue concentrations of empty microgels (IP), PAOPA only (IP) in PBS solution and exemplary PAOPA-loaded microgels

following intraperitoneal (IP) injection in rats at 10 mg/kg of PAOPA (n=2) in the prefrontal cortex (PFC), striatum and cerebellum. This was to determine whether the use of microgels led to greater PAOPA brain tissue concentrations after treatment. The use of an exemplary microgel drug carrier led to higher brain tissue concentrations than that of PAOPA injected in PBS solution alone.

DETAILED DESCRIPTION

I. Definitions

[0032] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present application herein described for which they are suitable as would be understood by a person skilled in the art.

[0033] In understanding the scope of the present application, the term "comprising" and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, "including", "having" and their derivatives. The term "consisting" and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The term "consisting essentially of", as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps.

[0034] Terms of degree such as "substantially", "about" and "approximately" as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least ±5% of the modified term if this deviation would not negate the meaning of the word it modifies.

[0035] As used in this application, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise. For example,

an embodiment including "a substance" should be understood to present certain aspects with one substance or two or more additional substances.

[0036] In embodiments comprising an "additional" or "second" component, such as an additional or second substance, the second component as used herein is chemically different from the other components or first component. A "third" component is different from the other, first, and second components, and further enumerated or "additional" components are similarly different.

[0037] The term "and/or" as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that "at least one of" or "one or more" of the listed items is used or present.

[0038] The term "microgel" as used herein refers to solvent-swollen three-dimensional cross-linked polymeric networks that exist as nano- or microsized particles

[0039] The term "chemically cross-linked microgel" as used herein refers to a microgel in which the polymer network is formed via covalent bonds.

[0040] The term "particle size" as used herein refers to the mean diameter of the microgel particles in a given microgel composition as determined using, for example, dynamic light scattering, or any other well-known technique for measuring this characteristic of a microgel.

[0041] The term "biodegradable" as used herein with reference to the microgels means that the polymer network will break down into sufficiently small fragments to allow renal clearance of the polymer within a time period sufficient to avoid accumulation of the microgel in a subject in amounts to cause undesirable effects. For example, biodegradability is introduced into a microgel by using cross-linkers that can undergo bond breaking (scission) within the physiological environment. Examples of such cross-linkers include, but are not limited to, acetals, activated esters, amides, anhydrides, disulfides, hydrazides, and/or imines. Incorporation of polysaccharides or other macromolecules that can be enzymatically cleaved within the physiological environment into microgels also promotes degradability (Cai T. et al. Langmuir, 2009, 23, 8663-8666).

[0042] The term "hydrophilic" as used herein refers to a property of a substance meaning that the substance has a tendency to interact with or be dissolved by water and other polar substances. A hydrophilic molecule or portion of a molecule is one that is typically polar and capable of hydrogen bonding, enabling it to dissolve more readily in water than in oil or other hydrophobic solvents.

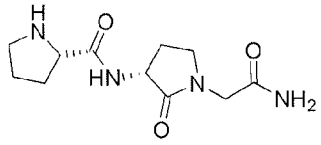
[0043] The term "hydrophobic" as used herein refers to a property of a substance meaning that the substance has a tendency to not interact with or be dissolved by water and other polar substances. A hydrophobic molecule or portion of a molecule is one that is typically nonpolar and not capable of hydrogen bonding, enabling it to dissolve more readily in oil or other hydrophobic solvents than in water.

[0044] The term "entrapped" as used herein means that the substance is located or entrapped within or on the polymer network of the microgel. The entrapment may be by physical means (i.e. encapsulation, where the polymer network forms a barrier around the molecule so that it cannot leave the network without some physical change in the network) or by chemical means (i.e. the molecule is covalently or electrostatically linked to the polymer network).

[0045] The term "biological targets" as used herein refers to biological molecules such as proteins, enzymes or receptors, in the brain, that the drug interacts with and modulates to cause a physiological effect, such as a beneficial physiological effect.

[0046] The term "modulates" as used herein refers to any change in the activity of a biological target due to interaction by the drug. Modulation of the biological target typically results in treatment of a disease, disorder or condition that benefits from a modulation the target

[0047] The term "PAOPA" as used herein refers to the compound 3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide having the following chemical structure:



[0048] The term "PAOPA derivative" as used herein refers a compound comprising the same chemical structure as PAOPA and other additional chemical moieties. For example, the PAOPA derivative is a salt, solvate and/or prodrug of PAOPA. In particular, the PAOPA derivative is a pharmaceutically acceptable salt, solvate and/or prodrug.

[0049] The term "PAOPA analog" as used herein refers to compound having a similar chemical structure as PAOPA or a PAOPA derivative, however certain atoms or or groups have been replaced with analogous atoms or groups. By "analogous" it is meant having a similar electronic or steric structure so that replacement by these atoms or groups does not detrimentally affect the activity of the PAOPA compound.

[0050] The term "pharmaceutically acceptable salt" means an acid addition salt or a basic addition salt suitable for, or compatible with, the treatment of subjects.

[0051] The term "pharmaceutically acceptable salts" embraces salts commonly used to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically-acceptable. Suitable pharmaceutically acceptable acid addition salts are prepared from an inorganic acid or an organic acid. Examples of such inorganic acids include, without limitation, hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Examples of appropriate organic acids include, for example, aliphatic, cycloaliphatic, aromatic, arylaliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include, without limitation, formic, acetic, propionic, succinic, glycolic, gluconic, maleic, embonic (pamoic), methanesulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, pantothenic, benzenesulfonic, toluenesulfonic, sulfanilic, mesylic, cyclohexylaminosulfonic, stearic, algenic, β-hydroxybutyric, malonic, galactic, and galacturonic acid. Suitable pharmaceutically-acceptable base addition salts include, but are not limited to, metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, lysine and procaine.

[0052] The formation of a desired compound salt is achieved using standard techniques. For example, the neutral compound is treated with an acid or base in a

suitable solvent and the formed salt is isolated by filtration, extraction or any other suitable method.

[0053] The term "solvates" as used herein refers to complexes formed between a compound and a solvent from which the compound is precipitated or in which the compound is made. Accordingly, the term "solvate" as used herein means a compound, or a salt a compound, wherein molecules of a suitable solvent are incorporated in the crystal lattice. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate". The formation of solvates will vary depending on the compound and the solvate. In general, solvates are formed by dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions. The selection of suitable conditions to form a particular solvate can be made by a person skilled in the art.

[0054] The term "pharmaceutically acceptable solvate" means a solvate suitable for, or compatible with, the treatment of subjects. For pharmaceutically acceptable solvates, a suitable solvent is physiologically tolerable at the dosage used or administered.

[0055] The expression "disease, disorder or condition that benefits from a modulation of targets in the brain" as used herein refers to any disease, disorder or condition that is directly or indirectly caused or affected by the target.

[0056] The term "subject" as used herein includes all members of the animal kingdom including mammals.

[0057] The term "pharmaceutical composition" as used herein refers to a composition of matter for pharmaceutical use.

[0058] The term "pharmaceutically acceptable" means compatible with the treatment of subjects, for example, mammals such as equines and humans.

[0059] The term "parenteral" as used herein means taken into the body or administered in a manner other than through the gastrointestinal tract.

[0060] The term "administered" as used herein means administration of an effective amount of a compound, including the antibiotic and compound of Formula I, or a salt and/or solvate thereof, to a cell either in cell culture or in a subject.

[0061] As used herein, the term "effective amount" or "therapeutically effective amount" means an amount effective, at dosages and for periods of time necessary, depending on the delivery route, to achieve a desired result. For example, in the context of treating a disease, disorder or condition that benefits from a modulation of targets in the brain, an effective amount of the composition(s) of the application, is an amount that, for example, reduces the disease, disorder or condition compared to the disease, disorder or condition without administration of the composition(s) of the application. Effective amounts may vary according to factors such as the disease state, age, sex and/or weight of the subject. The amount of a given composition that will correspond to such an amount will vary depending upon various factors, such as the given composition, the pharmaceutical formulation, the route of administration, the type of condition, disease or disorder, the identity of the subject being treated, and the like, but can nevertheless be routinely determined by one skilled in the art.

[0062] The terms "to treat", "treating" and "treatment" as used herein and as is well understood in the art, means an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results include, but are not limited to, diminishment of extent of the disease, disorder or condition, stabilization (i.e. not worsening) of the disease, disorder or condition, preventing spread of the disease, disorder or condition, delay or slowing of the disease, disorder or condition, amelioration or palliation of the disease, disorder or condition, diminishment of the reoccurrence of the disease, disorder or condition and remission of the disease, disorder or condition and/or one or more symptoms or conditions arising from the disease, disorder or condition, whether partial or total, whether detectable or undetectable. "To treat", "treating" and "treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "To treat", "treating" and "treatment" as used herein also include prophylactic treatment.

[0063] "Palliating" a disease, disorder or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the disease, disorder or condition.

[0064] The term "prevention" or "prophylaxis" and the like as used herein refers to a reduction in the risk or probability of a subject becoming afflicted with the

disease, disorder or condition or manifesting a symptom associated with the disease, disorder or condition.

[0065] When used, for example, with respect to the methods of treatment, uses, compositions and kits of the application, a subject, for example a subject "in need thereof" is a subject who has been diagnosed with, is suspected of having, may come in to contact with, and/or was previously treated for the disease, disorder or condition that benefits from a modulation of targets in the brain.

II. Compositions

[0066] The present application includes a microgel composition comprising:

(a) a chemically cross-linked microgel having a particle size of about 20 nm to about 300 nm; and

(b) one or more substances for delivery to the brain,

wherein the substance is entrapped in and/or on the chemically cross-linked microgel.

[0067] In an embodiment, the chemically cross-linked microgels comprise a polymer that is responsive to an external stimulus (a stimuli-responsive polymer), such as a chemical, biological, or physical stimulus, and sharply changes at least one of its physical properties in response to the stimulus. For example, the microgels comprise a bioresponsive polymer that responds to physical, chemical, or biological stimuli, such as temperature, pH, ionic strength, magnetic field, electrical field, pressure, light, enzyme, receptor, glucose, etc. by altering their swelling behavior, permeability or mechanical strength. Specifically, thermo-responsive polymers respond to environmental temperature changes with structural and morphological changes by absorbing or expelling water. In an embodiment, the stimuli-responsive polymer shows a lower critical solution temperature (LCST) that is close to mammalian body temperature. For example, the polymer may experience a sharp volume phase transition at the LCST, changing between hydrophilic at temperatures below the LCST and hydrophobic above the LCST. Additionally, pH-responsive polymers respond to environmental pH changes with structural and morphological changes by absorbing or expelling water. For example, the polymer can be designed to expel its contents at the pH where it is desired for the substance in the polymer to be released. Through copolymerizing and cross-linking with other hydrophilic or

hydrophobic monomers, the physical, chemical, and mechanical properties of the microgels can be tuned, adjusted and controlled to respond to different physiological stimuli.

[0068] In an embodiment, the chemically cross-linked microgels are stimuli-responsive and comprise polymeric units comprising oligo(ethylene glycol) acrylate (OEGA), oligo(ethylene glycol) methacrylate (OEGMA), oligo(ethylene glycol) diacrylate (OEGDA), *N*-alkylacrylamides (such as *N*-isopropylacrylamide (NIPAM) or N-isopropylmethylacrylamide (NIPMAM)), diethylene glycol (meth)acrylate ($M(EO)_2MA$), or vinyl caprolactone (VCL) units, or copolymers thereof.

[0069] In a further embodiment, the chemically cross-linked microgels are non-stimuli-responsive and comprise any such water-soluble polymer having a functional group that reacts with a linker group. In an embodiment, the water-soluble is selected from poly(ethylene glycol), poly(N-vinylpyrrolidone), poly(hydroxyethylmethacrylate) and carbohydrates (for example, carboxymethyl cellulose, hyaluronic acid, dextran, various glycosaminoglycans, and the like).

[0070] In an embodiment, these stimuli responsive or non-stimuli response polymers further comprise co-monomeric units, including, for example, (meth)acrylates, acrylates, acrylamides and vinyl carboxylic acids. In an embodiment, the co-monomeric units are selected from, acrylic acid (AA), fumaric acid (FA), vinyl acetic acid (VAA), methacrylic acid (MAA), butylmethylacrylate (nBuMA), dimethylaminoethyl acrylate (DMAEA), acetoacetoxymethacrylate, acrylamide (AAm), aminoethyl methacrylate (AEMA), dimethylaminoethyl (meth)acrylate (DMAEMA) and N-(3-aminopropyl)methacrylamide (APMA). In some embodiments, the co-monomeric units impart other types of stimuli-responses or reactive sites for drug, target, or other forms of grafting modification to the microgel.

[0071] In an embodiment the polymer units of the chemically cross-linked microgels are cross-linked with a linker group that is hydrolytically or enzymatically degradable under physiological conditions. In an embodiment the linker group comprises an acetal, activated ester, amide, anhydride, disulfide, hydrazide, and/or imine moiety. In a further embodiment, the chemically cross-linked microgel is cross-linked with a linker group comprising a disulfide. In an embodiment, the linker group

is selected to allow degradation of the microgel at the target site for delivery of the microgel *in vivo*.

[0072] In an embodiment, the chemically cross-linked microgels comprise chemically cross-linked POEGMA. In an embodiment, the chemically cross-linked microgels consist essentially of POEGMA cross-linked with a linker group comprising a disulfide.

[0073] In an embodiment of the application, the chemically cross-linked microgels are surface-functionalized with one or more molecules for cell targeting and/or internalization. In an embodiment, the one or molecules for cell targeting and/or internalization is selected from cell adhesive ligands (for example, fibronectin, vitronectin and laminin peptide sequences like RGD, YIGSR and the like), cadherins, integrins, glycoproteins, mucoadhesive ligands (for example, cationic polymers, poly(ethylene oxide), phenylboronic acids and derivatives, thiolated polymers, and the like) antibodies and aptamers. In an embodiment the one or more molecules for cell targeting targets the microgel to cells in the nasal cavity, for example, a lectin, in particular *Solanum tuberosum* lectin (STL) which targets the microgels to nasal epithelial cells.

[0074] In an embodiment the substance for delivery to the brain is a substance that modulates one or more biological targets in the brain. In a further embodiment, the substance that modulates one or more biological targets in the brain is an antipsychotic drug. In a further embodiment, the antipsychotic drug is selected from one or more of amantadine, amisulpride, apomorphine, aripiprazole, asenapine, blonanserin, bromocriptine, carbidopa, chlorpromazine, clotiapine, clozapine, divalproex, droperidol, entacapone, fluphenazine, haloperidol, iloperidone, levodopa, levomepromazine, lithium, loxapine, lurasidone, mesoridazine, molindone, mosapramine, olanzapine, PAOPA, paliperidone, perospirone, perphenazine, pramipexole, prochlorperazine, quetiapine, remoxipride, risperidone, ropinirole, selegiline, sertindole, sulpiride, thioridazine, thiothixene, tolcapone, trifluoperazine, valproic acid, ziprasidone, zotepine and zuclopenthixol, and analogs and derivatives thereof.

[0075] In an embodiment, the substance for delivery to the brain is a hydrophilic substance, such as a hydrophilic antipsychotic drug. In embodiment, the

hydrophilic antipsychotic drug is selected from one or more of amantadine, amisulpride, apomorphine, asenapine, bromocriptine, carbidopa, chlorpromazine, haloperidol, levodopa, levomepromazine, lithium, loxapine, lurasidone, molindone, mosapramine, prochlorperazine, quetiapine, remoxipride, ropinirole, selegiline, sertindole, sulpiride, thioridazine, thiothixene, tolcapone, trifluoperazine, valproic acid, zotepine and zuclopenthixol, and analogs and derivatives thereof.

[0076] In a further embodiment, the antipsychotic drug is PAOPA or a PAOPA analog or derivative.

[0077] In an embodiment the substance for delivery to the brain is a substance that is used to image areas of the brain. In a further embodiment, the substance used to image areas of the brain (brain imaging agents) is selected from one or more of MRI imaging agents (for example, gadolinium- or iron-based imaging agents, such as gadopentetate dimeglumine, nitroxides, gadobenate dimeglumine, gadobutrol, gadodiamide, gadoterate meglumine, gadoteridol, gadoversetamide, gadoversetamide, metallofullerines), fluorescent markers (for example, quantum dots, fluorescein and derivatives, rhodamine and derivatives, and the like) and radioactive imaging agents (for example, I-125, I-131, I-132, Tc99m and Mo99) .

[0078] In a further embodiment, the chemically cross-linked microgels are further chemically conjugated with a layer of hydrophilic polymer, for example, to reduce opsonin adsorption. In an embodiment the hydrophilic polymer is polyethylene glycol (PEG).

[0079] In a specific embodiment of the present application there is included a microgel composition comprising (i) a water-swollen POEGMA polymer network cross-linked via disulfide bonds and (ii) PAOPA.

[0080] In an embodiment the chemically cross-linked microgels are prepared using free radical polymerization in the context of precipitation polymerization. Such methods are known in the art. Such methods are used, for example, to prepare microgels comprising stimuli-responsive polymers. In a further embodiment, the chemically cross-linked microgels are prepared using an inverse emulsion method, wherein a water-in-oil emulsion is used as a template to make the particle. Such

methods are used, for example, to prepare microgels comprising non-stimuli-responsive polymers.

[0081] In a further embodiment, surface functionalization is performed on the chemically cross-linked microgel by reaction with surface functional groups, such as external carboxylic acids (e.g. acrylic acids) with a nucleophile, such as an amino, thio or hydroxyl group on the molecule to be conjugated with the microgel, in the presence of a coupling reagent. Methods of preparing and surface functionalizing microgels are known in the art.

[0082] The proportion of the substance(s) incorporated in the microgels of the application can vary within a wide range. Nevertheless, the suitable proportion will depend in each case on the substance incorporated and its intended use.

[0083] In an embodiment, pharmaceutical or diagnostic compositions are prepared with the microgel compositions of the invention. The pharmaceutical or diagnostic compositions are useful, for example, for delivering the substances(s) encapsulated therein to the brain. Accordingly, in another embodiment, the present application includes a pharmaceutical or diagnostic comprising one or more of the microgel compositions of the application and a pharmaceutically acceptable carrier, diluent and/or adjuvant.

[0084] In an embodiment, the pharmaceutical or diagnostic compositions comprising one or more microgel compositions of the application are administered to a biologically system or organism. In one embodiment, the pharmaceutical or diagnostic compositions comprising one or more microgel compositions of the application are administered to a subject in need of receiving such a composition. As used herein, a subject is any living organism including a human and administrating to the subject comprises, without limitation, inserting, injecting, implanting, delivering, and infusing the composition in or to the subject. In an embodiment, the administration is local administration, oral administration, intraperitioneal administration, systemic administration, intravenous administration, transdermal administration, intramuscular administration, intra/extravascular administration, intra-arterial administration, intrathecal administration, intracranial administration, conjunctival administration, intra-amniotic administration, chemotherapeutic administration, rectal administration, ophthalmic administration, nasal administration,

percutaneous administration, or subcutaneous administration. In an embodiment of the present invention, a human subject is administered a pharmaceutical or diagnostic compositions comprising one or more microgel compositions of the application wherein the substance encapsulated in the microgel is administered to have a concentration of up to approximately 1,000 mg/mL.

[0085] In another embodiment, the pharmaceutical or diagnostic compositions comprising one or more microgel compositions are administered as a drug or imaging agent delivery vehicle to a subject in need thereof. The compositions can release the substance(s) in a controlled sustained manner for a few hours to up to several days and even years and up to a release rate of about 0-10 g/day, e.g. about 1x 10^{-6} g to about 1 g/day.

[0086] In an embodiment, the microgel compositions are administered to the subject, or used, by oral (including sublingual and buccal) or parenteral (including, intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, topical, patch, pump and transdermal) administration and the microgel compositions formulated accordingly. Conventional procedures and ingredients for the selection and preparation of suitable compositions are described, for example, in Remington's Pharmaceutical Sciences (2000 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[0087] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form is sterile and fluid to the extent that easy syringability exists.

[0088] Compositions for nasal administration are conveniently formulated as aerosols, drops, gels or powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which take the form of a cartridge or refill for use with an atomising device. Alternatively, the sealed container is a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form

comprises an aerosol dispenser, it contains a propellant which is, for example, a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. In an embodiment, the aerosol dosage forms take the form of a pump-atomizer.

[0089] Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, wherein the active ingredient is formulated with a carrier such as sugar, acacia, tragacanth, gelatin and/or glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base such as cocoa butter.

[0090] In another embodiment, the microgel compositions are orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it is enclosed in hard or soft shell gelatin capsules, or it is compressed into tablets, or it is incorporated directly with the food of a diet. For oral administration, the microgel compositions are incorporated with excipients and used in the form of, for example, ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

[0091] It is also possible to freeze-dry the microgel compositions and use the lyophilizate obtained, for example, for the preparation of products for injection.

III. Methods and Uses

[0092] In another embodiment, the present application includes a method of treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain comprising administering, to a subject in need thereof, an effective amount of one or more microgel compositions of the application, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain.

[0093] The present application also includes a use of one or more microgel compositions of the application for treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain. Also included is a use of one or more microgel compositions of the application for preparation of a medicament for treating a condition, disease

and/or disorder that benefits from a modulation of targets in the brain, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain. Further includes is a microgel composition of the present application for use in treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain, wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain.

[0094] Treatment methods comprise administering to a subject the microgel compositions of the application, or pharmaceutical compositions comprising the microgel compositions of the application, and optionally consists of a single administration, or alternatively comprises a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the disease, disorder or condition, the age of the subject, the dosage of the substance, the activity of the substance, and/or a combination thereof.

[0095] It is an embodiment that that substance is administered or used according to a treatment protocol that is known for the substance in treating the disease, disorder or condition.

[0096] The dosage of the substance, and accordingly the microgel compositions of the application, varies depending on many factors such as the pharmacodynamic properties thereof, the mode of administration, the age, health and weight of the subject, the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any, the activity of the substance, the timing of the release and the release rate of the substance from the microgel and the clearance rate in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. The substance, and accordingly the microgel compositions of the application, may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response.

[0097] In another embodiment, the present application includes a method of imaging the brain comprising administering, to a subject in need thereof, an effective amount of one or more microgel compositions of the application, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain, and performing an imaging method on the subject.

[0098] The present application also includes a use of one or more microgel compositions of the application for imaging the brain, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain. Also included is a use of one or more microgel compositions of the application for preparation of a medicament for imaging the brain, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain. Further includes is a microgel composition of the present application for use in imaging the brain, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain.

III. Other Embodiments

[0099] In embodiments of the application, there is included a functionalized microgel network comprised of water-swollen poly [oligo(ethylene glycol) methyl ether methacrylate] (*POEGMA*) networks with the following characteristics: <150 nm in size with a very low polydispersity, very low in surface charge (<20 mV), biodegradable through the use of a cross-linker containing disulfide bonds that can selectively be cleaved in the presence of cellular glutathione.

[00100] In a further embodiment, the microgel network is surface functionalized with STL or any other particle surface functionalization type.

[00101] In a further embodiment the microgel network encapsulates a hydrophilic drug.

[00102] In a further embodiment, the microgel network encaopsulates PAOPA (3(R)-[(2(S)-pyrrolidinylcarbonyl)amino] -2-oxo-1-pyrrolidineacetamide).

[00103] In a further embodiment, there is included a process of manufacturing the functionalized microgels of the application comprising the steps of precipitation polymerization in water in the presence of OEGMA monomers, acrylic acid, and a disulfide-containing cross-linker at 90°C to create the POEGMA microgel and the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to react acrylic acid residues with terminal amino groups present on the functionalization unit (ex. *Solanum tuberosum* lectin) for labeling.

[00104] In a further embodiment, there is included a method of treating a dopamine-related neuropsychiatric disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a microgel encapsulating PAOPA wherein the network is a functionalized microgel network comprised of water-swollen poly [oligo(ethylene glycol) methyl ether methacrylate] (*POEGMA*) networks with the following characteristics: <150 nm in size with a very low polydispersity, very low in surface charge (<20 mV), biodegradable through the use of a cross-linker containing disulfide bonds that can selectively be cleaved in the presence of cellular glutathione and is optionally is surface functionalized with STL or any other particle surface functionalization type.

[00105] In an embodiment, the dopamine-related neuropsychiatric disorder is selected from the group consisting of schizophrenia, autism spectrum disorders (ASD), bipolar disorder, depression, Alzheimer's disease, Lewy body disease, Parkinson's disease and drug or behavioural addictions.

[00106] In a further embodiment, the microgel network is administered orally, subcutaneously, intravenously, intraperitoneally, intranasally, enterally, topically, sublingually, intramuscularly, intra-arterially, intramedullarly, intrathecally, by inhalation, ocularly, transdermally, vaginally or rectally.

EXAMPLES

The following non-limiting examples are illustrative of the present application:

Methods

POEGMA microgel synthesis

[00107] POEGMA microgels were synthesized via precipitation polymerization in water at 90°C. Diethylene glycol methacrylate (1.5 g, 8.0 mmol), 2-hydroxyethyl disulfide dimethacrylate (90 µL, 0.3 mmol), acylic acid (528 µL, 7.7 mmol) and sodium dodecyl sulfate (60 mg, 0.21 mmol) were added to a two-neck round bottom flask and distilled deionized water (145 mL) was added. The resulting emulsion was purged with nitrogen under continuous agitation for at least 30 minutes prior to heating to the reaction temperature of 90°C. A solution of potassium persulfate (60 mg, 0.22 mmol in 5 mL distilled deionized water) was added instantaneously to start

the polymerization. The polymerization was quenched after 4 hours by cooling and exposing to air. The microgel suspension was subsequently dialyzed against distilled deionized water for at least 4 cycles of 6 hours each. Particle size analysis was performed on a Malvern Zetasizer Nano-ZS at a concentration of 10 mg/mL in phosphate buffered saline (PBS) at pH = 7.4. The acrylic acid content was determined by titration with sodium hydroxide using a PC-Titrate system (Mandel Scientific Co., Canada).

POEGMA microgel surface functionalization

[00108] The POEGMA microgels were subsequently surface-functionalized with lectins via an 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) reaction between carboxylic acids of the external acrylic acids and the terminal amine of the STL. Each sample to be functionalized contained 25 mg of POEGMA, which was transferred into 3 ml ultracentrifuge tubes and subjected each to 30,000 rpm for 45 min (4°C) using an Optima TL-100 Ultracentrifuge (Beckman Coulter, USA). The supernatant was removed and samples were placed on ice to ensure particle stabilization. Samples were also place on ice intermittently during the pellet resuspension to encourage reconstitution. The coupling procedure (molar ratio of STL:acrylic acid = 1:10,000; molar ratio of EDC:acrylic acid = 10,000:1) was performed in 20 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 5.05) on a rocking platform at room temperature overnight. Samples were then transferred to 3 mL ultracentrifuge tubes and subjected to 30,000 rpm for 45 min (4°C) in using an Optima TL-100 Ultracentrifuge (Beckman Coulter, USA). The supernatant was then collected to determine STL conjugation efficiency to the microgel surface acrylic acid residues using a standard curve with the spectrophotometer.

POEGMA and STL-POEGMA microgel drug loading

[00109] A 25 mg solution of either POEGMA or STL-functionalized POEGMA microgels were transferred to 3 mL ultracentrifuge tubes and subjected to 30,000 rpm for 45 min (4°C) using an Optima TL-100 Ultracentrifuge (Beckman Coulter, USA). Following this, the supernatant was removed and the microgels were reconstituted in 2 mL of PBS (pH 7.4) with 3.5 mg PAOPA. These samples were then transferred to 15 mL Falcon tubes and put on a GyroMini Nutating Mixer (Labnet International Inc., USA) for 1-2 days. Once complete, the drug-loaded samples were

placed into ultracentrifuge tubes and subjected to 30,000 rpm for 45 min (4°C) using an Optima TL-100 Ultracentrifuge (Beckman Coulter, USA). The supernatant from each sample was then collected for drug-loading measurements via HPLC (see below) and the sample pellets were resuspended in 1 mL PBS solution (pH 7.4).

Microgel characterization, concentration and lectin conjugation density

[00110] The mean diameter of the microgels was determined by dynamic light scattering (DLS) analysis at 37°C in PBS (pH 7.4) using the Malvern Zetasizer Nano-ZS (Malvern, UK). The zeta potential of the microgels was measured at 37°C in PBS (pH 7.4) using the Zeta 90 Plus Zeta Potential Analyzer (Brookhaven Instrument Corp., USA). The morphological examination of the nanoparticles was performed using a JEM-1200ex transmission electron microscope (TEM) (Peabody, USA). Microgel concentration was determined using nanoparticle tracking analysis (NTA) with a LM 20 NanoSight (Wiltshire, United Kingdom). The quantity of STL conjugated to the surface of the microgels was determined by measuring the concentration of unconjugated STL within the supernatant following the centrifugation step. The conjugation efficiency was calculated using the equations described by Chen *et al.*, 2011:

Conjugation density = (Amount of STL (mol/mL) x Avagadro's Number x conjugation efficiency)/Total nanoparticle concentration per mL

Conjugation efficiency = [(Amount of STL added - Amount of unconjugated STL)/ Total amount of STL added] × 100%

In vitro release of PAOPA from POEGMA and STL-POEGMA microgels

[00111] *In vitro* release experiments of PAOPA from microgels were performed at 37°C in PBS (10 mM, pH 7.4) to evaluate the APD leakage over a 96 h (5 day) incubation period. The pH 7.4 phosphate buffer was selected to represent the pH of the cellular and extracellular compartment. The entire batch of non-functionalized or STL-functionalized PAOPA-loaded microgels suspended in 1 mL of PBS (10 mM, pH 7.4) was added to the internal chamber of the Float-a-Lyzer G2 dialysis device (Sigma-Aldrich, Oakville, ON) and the external chamber of the device was filled with 6 ml of PBS (10 mM, pH 7.4). The samples were placed into a beaker in an incubator at 37°C at 100-120 rpm after closing the Float-a-Lyzer. At given time

intervals (2, 4, 6 , 8, 12, 24, 48, 72, 96 hrs) 2 mL aliquots were removed for high performance liquid chromatography (HPLC) analysis (see below) and the removed volume was replaced with fresh 2 mL of PBS (10 mM, pH 7.4).

Determination of encapsulation efficiency, drug loading capacity, and in vitro PAOPA release

[00112] The amount of PAOPA encapsulated was quantified via HPLC by difference, measuring the total loss of PAOPA at each step during microgel loading as opposed to after synthesis. This approach avoids requiring complete dissolution of the drug loaded nanoparticles to measure loading, which due to solvent incompatibilities in dissolving the polymer and the drug can result in significant overestimates in drug loading (Budhian, Siegel, & Winey, 2005). The microgel drug entrapment efficiency and drug loading capacity of PAOPA was determined using gradient HPLC. A sample injection volume of 20 µL was used for PAOPA samples, measured using a Waters 2695 separation module (Model SM4; Waters Corporation, Canada) consisting of a Waters 2489 ultraviolet detector (Model 246; λ = 215 nm for PAOPA). PAOPA was run using the Xbridge (C18) (5 µm, 150 mm × 4.6 mm), using a column temperature of 35°C and a flow rate of 1 mL/min. The separations for PAOPA were achieved using a gradient of mobile phase A (100% Water) and mobile phase B (100% Acetonitrile). The gradient was composed of 80:20 v/v (mobile phase A:mobile phase B) to 20:80 v/v (mobile phase A:mobile phase B). The drug loading capacity (DLC) and encapsulation efficiency (EE) were calculated as follows:

$$DLC\ (\%) = \frac{C \times V}{M \times 1000} \times 100\% \qquad EE\ (\%) = \frac{C \times V}{T} \times 100\%$$

where C = concentration of drug encapsulated, V = volume of diluted microgels, M = total microgel weight, and T = amount of drug added.

POEGMA and STL-POEGMA microgel degradation

[00113] Either a 20 mg/ml solution of POEGMA or STL-functionalized POEGMA microgels were subjected to ultracentrifugation at 30,000 rpm for 45 min (4°C) using an Optima TL-100 Ultracentrifuge (Beckman Coulter, USA). Following this, the supernatant was removed and the microgels were reconstituted in glutathione (GSH) in PBS (10 mM, pH 7.4). The microgel suspensions were incubated at 37°C for 12 hrs and then visually inspected for degradation.

PAOPA, POEGMA and STL-POEGMA microgel cytotoxicity studies

[00114] MTT assays were performed in order to evaluate the cytotoxicity of the drug PAOPA, POEGMA and STL-POEGMA microgels to the RPMI 2650 Nasal Septum Carcinoma as well as SH-SY5Y dopamine D2 receptor transduced neuronal cell lines, performed using a modified form of the assay manufacturer's protocols as described by Pawlikowska *et al.*, 2006. This was to determine whether the drug or drug carriers will be cytotoxic after administration in the nasal cavity or following entry into the brain. Approximately 4 x 10^4 cells/mL were plated in each well of a 96 well plate and the final volume of each of the wells of the 96 well plate was brought up to 150 µL. Once the cells were 90-95% confluent (after 2-3 days) and had adhered to the bottom of the wells, the media was discarded into bleach. Five different treatment concentrations (400, 800, 1200, 1600 or 2000 mg/mL) with an n=4 wells for each treatment concentration were given for each treatment type. The treatment types were either: a) media only (for blank calculation), b) haloperidol (positive control), c) PAOPA, d) POEGMA microgels, or d) STL-POEGMA microgels. After 24 hours of treatment exposure, the treatment/media was removed and 0.5 mg/mL MTT was added to each well. The cells were incubated for 3-4 hours in incubator at 37°C and following the incubation, the MTT was removed from each well. To each well 100 µL dimethyl sulfoxide (DMSO) was then added to solubilize the formazan precipitate. The place was then incubated for 15 min on an Orbital Shaker (Bellco Biotechnology, USA) and the samples were read on a Synergy 4 microplate reader (Biotek, USA). The absorbance was determined as the difference between the measurement wavelength of 570 nm and the reference wavelength of 690 nm. The cell viability percentages were calculated as outlined in the formula below.

$$Cell\ Viability(\%) = \frac{Absorbance_{Polymer\ Solution}}{Absorbance_{Blank}}$$

Rhodamine-labelled POEGMA microgel treatment, perfusion and tissue extraction

[00115] Animals were first put under gaseous anaesthesia using isofluorane after 1 hour following IP injection of one of three sizes of rhodamine-labelled microgel (non-labelled microgels, 100 nm, 150 nm and 250 nm). On the wet table in the fume hood, the animals were pinned down to the board with their noses in the gas cone to remain anaesthetized. The chest cavity was opened up by cutting each side of

the rib cage to expose the still beating heart. A vacuum tube attached to an adaptor and butterfly clip were used to draw blood from the right ventricle into acid-citrate-dextrose (ACD) tubes to collect blood samples. A butterfly clip with needle was used to puncture the left ventricle and was clamped to the skin to first remove all of the blood by injecting saline using a 60 ml syringe. Once the fluid escaping the vasculature had run clear, the solution was switched to 4% paraformaldehyde (PFA) in PBS (10 mM, pH 7.4) to 'fix' the tissues. When the tissues lost most of their color, the animals were then decapitated. The tissues (i.e. brain, liver, lungs, spleen and kidneys) were removed and placed in 4% PFA overnight to 'post-fix' the tissues. The tissues were then rinsed in 4% PFA and placed into 20% sucrose. When the tissues had sunk, a brain mold was used to excise the PFC, striatum and cerebellum. All of the tissues were then ready for homogenization.

Rhodamine-labelled POEGMA and STL-POEGMA microgel tissue uptake studies

[00116] The extracted brain and major organ tissues were first cut as much as possible using a sharp set of scissors and then homogenized in 1 mL of anhydrous ethyl alcohol (2 x 30s intervals). The homogenized tissues were vortexed and then subjected to centrifugation at 9500 RPM for 5 min using a 5415 R, 230 V/50-60 Hz centrifuge (Eppendorf, Germany). The supernatant from each was transferred to new eppendorf tubes for analyses and stored at -20°C until analysis. Using a Cary Eclipse fluorometer (Varian Medical Systems, USA) with a 1 mL quartz cuvette and a calibration curve for each of the three microgel samples sizes (100, 150 and 250 nm), the microgel sample tissue concentrations were measured (excitation = 488 nm, emission = 590 nm of rhodamine 6G).

PAOPA-loaded POEGMA microgel treatment, tissue extraction, and tissue analysis

[00117] Animals were first put under gaseous anaesthesia using isofluorane after 1 hour following IP injection either empty microgels (i.e. not drug loaded), PAOPA in PBS solution, or PAOPA-loaded microgels. All animals receiving PAOPA were given a dose of 10 mg/kg. At this point the animals were euthanized and three brain regions were extracted (PFC, striatum and cerebellum). The extracted brain regions were first cut as much as possible using a sharp set of scissors and then homogenized in 3 volumes of 1.15% KCl solution (2 x 30s intervals). After this 2 volumes of 100% HPLC grade methanol was added to each sample to precipitate

proteins present in the homogenized tissues, samples were votexed and then subjected to centrifugation at 9500 RPM for 5 min using a 5415 R, 230 V/50-60 Hz centrifuge (Eppendorf, Germany). The supernatant from each was then filtered into HPLC vials using Acrodisc 25 mm syringe filters (0.22 μm) (Pall Corporation, USA) and run using the HPLC procedures described above for PAOPA tissue concentration.

Results

POEGMA microgel characterization, concentration and lectin conjugation density

[00118] The size of all microgel formulations was measured to ensure that the nanoparticles would be small enough to cross the BBB via passive transport. All functionalized and non-functionalized nanoparticles were found to have a z-average diameter of <150 nm (Table 1). There were no significant differences in particle size as a function of particle STL-functionalization (t=2.633, d.f.=2, P=0.119) (Table 1). The polydispersity index (PDI) from DLS ranged between 0.13~0.24 and suggesting a reasonably narrow distribution of particle sizes. The TEM showed spherical particle morphology with slightly smaller particle size than that observed via DLS due to the required drying of the microgels to perform TEM analysis (Figure 2). Nanoparticle tracking analysis indicated a nanoparticle concentration of 3.3-3.6 x 10^{12} particles/mL per microgel preparation. All measures of zeta potentials ranged between −13 to −17 mV, with the charge originating from the acrylic acid residues at the particle interface following microgel synthesis and the net negative charge on the lectin targeting ligand. The presence of this STL functionalization had a significant effect on increasing surface charge (t=1.55, d.f.=4, P=0.0194) as a result of the residual negative charge on the lectins. The spectrophotomometric analysis showed that a STL conjugation efficiency of 85.5 ± 1.6 % was achieved, resulting in a surface density of STL on the nanoparticles of 429.8 ± 7.9 STL/microgel. The 10 fold excess of EDC to microgel acrylic acid content (10:1 molar ratio) was chosen in order to maximize the efficacy of the EDC reaction when attempting to functionalize the microgel surface for *N*-acetylglucosamine residue targeting on the cell surface.

Drug quantification and release from functionalized and non-functionalized POEGMA microgels

[00119] The encapsulation efficiency was measured to determine the ratio of the weight of drug incorporated into the microgels relative to the total weight of drug added. Drug loading capacity was measured to determine the ratio of drug to the weight of total carrier system. Table 2 indicates that all drug loaded formulations have reasonably high DLCs and EEs, which demonstrates the efficacy of the nanoparticle preparation process described. Microgel functionalization was not found to have a significant effect on EE or DLC ($t=1.127$, d.f.=4, $P>0.05$). There was a slight decrease in EE for both drug-loaded nanoparticles following STL functionalization, indicative of the loss of some the POEGMA microgels during the additional processing steps involved in the STL functionalization process prior to drug loading. However, no significant drug leaching appears to occur from the nanoparticles that are retained during the STL functionalization process (Table 2).

In vitro drug release from STL-functionalized and non-functionalized PAOPA-loaded POEGMA microgels

[00120] The rate of *in vitro* release of the total loaded amount of PAOPA in physiological conditions is fairly slow over 96 hours (demonstrating minimal drug leakage and the potential for efficient drug transport to the targeted brain tissue). As demonstrated in Figure 3, the total observed *in vitro* release for PAOPA in pH 7.4 phosphate buffer at 37 °C was 100% of loaded drug from both the STL-microgels and the non-functionalized microgels only after a 96 hr incubation period (indicating the potential for complete drug release over extended periods of time). No significant difference was observed in drug leaching between non-functionalized over STL-functionalized PAOPA-loaded microgels (Figure 3). This result suggests that minimal drug is lost from the microgels during the period over which they would be transported to the target tissue (i.e. over the course of 2-4 hours).

POEGMA and STL-POEGMA microgel degradation experiments

[00121] The microgel suspension, which appeared translucent, went opaque upon the addition of GSH. After incubating for 12 hours, the microgels had degraded and a clear solution was obtained with a polymer precipitate (Figure 4).

Effect of PAOPA, POEGMA and STL-POEGMA microgels on cellular viability

[00122] No significant decrease in cell viability was noted for RPMI 2650 Nasal septum carcinoma cells after 24 h of treatment with the PAOPA, POEGMA microgel, or STL-POEGMA microgel treatments compared to the untreated control cells (Figure 5). Though there was a slight concentration dependent decrease in cell viability with increasing treatment concentrations, only a 10-15% decrease in cell viability was observed at exceptionally high treatment concentrations (i.e. 2 mg/mL). Similarly, for SHS 5Y neuronal cells, minimal decrease in cellular viability was observed after 24 h of treatment with the PAOPA, POEGMA microgel or STL-POEGMA microgel treatments compared to the untreated control cells (Figure 6). A similar, extremely small 10-15% decrease in cell viability was observed but only at exceptionally high treatment concentrations (i.e. 2 mg/mL), indicating good cytocompatibility of the microgel formulations.

Tissue analysis following intraperitoneal administration of rhodamine-labeled POEGMA microgels

[00123] Microgels were labeled via copolymerization with a small fraction (4.5 mg) of a rhodamine methacrylate monomer. The microgel sizes, PDIs and particle concentrations were determined as that of the other POEGMA microgels (see Table 3). As previous, microgels could be produced within the <150nm size range desirable for BBB transport with low polydispersities.

[00124] The brain tissue concentrations of IP administered rhodamine-labeled microgels of three different sizes were also measured within the brain (PFC, striatum and cerebellum) and the major organs (liver, lungs, spleen and kidneys. Figure 7 shows that there was a significant difference in rhodamine-labelled microgel tissue concentration across the different tissue types (ANOVA: $F_{(3, 48)} = 28.7$, $p < 0.0001$). All between groups comparisons between non-rhodamine labelled 100 nm microgels and rhodamine-labelled microgels were significant for all tissue samples ($p<0.001$). There was a significant between groups difference in the 100 nm and 150 nm rhodamine-labelled microgel concentration within the prefrontal cortex ($t = 2.734$, $p < 0.05$). There was also a significant between groups difference in the 100 nm and 150 nm rhodamine-labelled microgel concentration within the striatum ($t = 5.084$, $p < 0.001$). However, there was no between groups difference in the 100 nm and 150 nm rhodamine-labelled microgel cerebellum concentrations following IP injection ($t =$

1.846, p> 0.05). There was a significant between groups difference in the 100 nm and 250 nm rhodamine-labelled microgel concentration within the striatum (t = 3.59, p< 0.01). There was no significant between groups difference in the 100 nm and 250 nm rhodamine-labelled microgel concentration within the PFC or cerebellum (t = 2.033, p>0.05; t = 1.169, p>0.05). There was a significant between groups difference in the 150 nm and 250 nm rhodamine-labelled microgel concentration within the striatum (t = 4.230, p<0.01), but not the PFC, striatum or cerebellum (t = 0.7005, p>0.05; t = 0.6765, p>0.05). Though many of the between groups comparisons within the brain were not significant, there was a significant difference between the non-labelled microgels and the rhodamine-labelled microgels regardless of microgel size (ANOVA: $F_{(3, 44)} = 28.70$, p< 0.0001). Figure 7 shows a trend in that the administration of smaller microgels results in greater brain tissue uptake in all three regions.

[00125] Figure 8 shows that all between groups comparisons between non-rhodamine labelled 100 nm microgels and rhodamine-labelled microgels were significant for all tissue samples (p<0.001). Though none of the between groups comparisons within the major organs were significant based upon rhodamine-microgel size (p>0.05 in every instance), but there was a significant difference between the non-labelled microgels and the rhodamine-labelled microgels regardless of microgel size (ANOVA: $F_{(3, 48)} = 12.54$, p< 0.0001). Figure 8 may suggest a trend in that the administration of smaller microgels results in greater major organ tissue uptake in all four organs.

Tissue analysis following intraperitoneal administration of PAOPA-loaded POEGMA microgels:

[00126] The brain tissue concentrations of PAOPA were measured following IP administration of empty microgels, PAOPA only in PBS solution and PAOPA-loaded microgels within the several brain regions (PFC, striatum and cerebellum). Figure 9 shows that there was a significant difference in PAOPA tissue concentration across the different tissue types (ANOVA: $F_{(2, 36)} = 6.64$, p< 0.05, p=0.0169). There was a significant between groups differences between the empty microgels and PAOPA only in PBS solution as well as between the empty and PAOPA-loaded microgels in PAOPA concentrations within the PFC (t = 9.682228, p< 0.001; t =

13.81079, $p < 0.001$). In addition, there was also a significant between groups difference between the PAOPA only in PBS and PAOPA-loaded microgels in PAOPA concentrations within the PFC ($t = 4.128556$, $p < 0.01$). There was a significant between groups differences between the empty microgels and PAOPA only in PBS solution as well as between the empty and PAOPA-loaded microgels in PAOPA concentrations within the striatum ($t = 10.55510$, $p < 0.001$; $t = 15.55182$, $p < 0.001$). In addition, there was also a significant between groups difference between the PAOPA only in PBS and PAOPA-loaded microgels in PAOPA concentrations within the striatum ($t = 4.996717$, $p < 0.001$). PAOPA only in PBS solution as well as between the empty and PAOPA-loaded microgels in PAOPA concentrations within the cerebellum ($t = 8.945856$, $p < 0.001$; $t = 10.77356$, $p < 0.001$). There was no significant between groups difference between the PAOPA only in PBS and PAOPA-loaded microgels in PAOPA concentrations within the cerebellum ($t = 1.827704$, $p > 0.05$).

Discussion

[00127] Small particle size (<150 nm) is desirable for passive transport across the BBB through the endothelial cell tight junctions lining the ethmoidal capillary walls, which deny entry to 98% of all potential CNS therapeutics (Veisch, Gunn, & Zhang, 2010). The compositions reported herein for PAOPA (non-functionalized and STL-functionalized) could easily be fabricated to be <150 nm in size and as low as 80 nm in diameter, which is generally smaller than that of other reported APD-loaded nanoparticle preparations from the literature (Benvegnú *et al.*, 2011; Budhian, Siegel, & Winey, 2005; Budhian, Siegel, & Winey, 2008; Muthu & Singh, 2007; Muthu & Singh, 2008).

[00128] Contrary to some previously reported results in other systems, the loading of PAOPA slightly decreased the size of the POEGMA microgels. While not wishing to be limited by theory, this was likely due to electrostatic quenching of microgel-bound anionic groups (from acrylic acid residues) engineered into the microgel by the cationic PAOPA molecule, leading to higher uptakes and lower release rates than non-functionalized microgels.

[00129] The particle design for both formulations used herein assists in avoiding nasal clearance mechanisms by preventing nanoparticle-mucin interaction at

the nasal mucosa due to low-surface charge, which results in mucosa penetration rather than particle aggregation and mucus adhesion followed by clearance via beating nasal cilia (Illum, 2002; Singh & Lillard, 2009). The microgels displayed a low negative surface charge regardless of STL-functionalization, meaning that the particles should not readily aggregate upon contact with the nasal mucosa and that both carriers should be non-immunogenic due to the prevention of non-specific binding of opsonins to the particle surface for macrophage targeting (Gao *et al.*, 2006; Smeets & Hoare, 2013). No significant particle aggregation in solution was observed until even several weeks to a month after synthesis/drug loading, this particle stability can be regarded as favorable for nasal epithelial and brain endothelial cell transport (Gao *et al.*, 2006; Muthu & Singh, 2008) to enable microgel penetration first into the bloodstream and then into the brain.

[00130] While not wishing to be limited by theory, the strong association leading to the moderate PAOPA encapsulations achieved herein may be attributed to the anionic interactions between the –COOH-terminal of the acrylic acid residues, anionic at physiological pH, and the secondary amine group present in PAOPA, which is cationic at physiological pH. Note that encapsulation efficiencies in microgel formulations depend strongly on the concentration of the drug added to the microgel in the adsorption step, such that results in the literature are not directly comparable. The loadings achieved herein are acceptably high for clinical use.

[00131] The rate of *in vitro* release of the loaded amount of PAOPA in physiological conditions (pH 7.4 at 37°C) is fairly slow, with only 12-17% of drug released after 2 hours (typical of transport times of nanoparticles to the brain), demonstrating minimal drug leakage and the potential for efficient drug transport to its allosteric molecular target. 100% release of the drug was achieved over 96 hours, indicating that drug can escape from the carrier highly effectively following sequestering in the brain to yield a clinical effect. This result suggests that most of the PAOPA detected in cell or tissue samples would be attributable to the drug delivered locally by their drug carriers instead of non-specific, systemically circulating drug (Chen *et al.*, 2011; Gao *et al.*, 2006). The efficacy of the present compositions in retaining the encapsulated drug is beneficial to minimize drug leakage from the microgels before they reach the desired target of interest (in the present case, the

dopamine D2 receptor) and may allow for the drug to stay in the system longer than the 6 hr half-life of PAOPA thereby improving the steady state level (20 mL/min/kg) following IV administration (Tan *et al.*, 2013).

[00132] None of the treatments (PAOPA, POEGMA, or the STL-POEGMA microgels) were found to have any detrimental effect on cellular viability in any cell line tested. This is the first case in which POEGMA polymers have been examined for their cytoxicity within RPMI 2650 nasal septum or SHS 5Y neuronal carcinoma cells and suggests that this formulation will not cause any kind of tissue damage in nasal cavity during intranasal administration or within the neuronal cells themselves following passage across the blood brain barrier.

[00133] Before attempting to administer PAOPA-loaded microgels using any mode of administration, it was desirable to determine whether the POEGMA microgels were capable of passing the BBB following intranasal administration as this has never been previously demonstrated. In order to test whether the size of the microgels may affect its ability to cross the BBB, rats were given an IP injection of differing sizes of rhodamine-labelled microgels and brain region-specific concentrations were analyzed. The regions chosen were the PFC due to presence of the D2S in the mesocortical pathway, the striatum, which contains one of the highest D2 receptor concentrations within the brain, and the cerebellum due to the deep projections of dopaminergic neurons into the cerebellar cortex from the VTA (Bertolino *et al.*, 2009; Chen *et al.*, 2011; Ikai *et al.*, 1992; Weiner & Brann, 1989). PFC, striatal and cerebellum concentrations of 100 nm rhodamine-labelled microgels were shown to be higher than that of the other two microgel sizes due to its easier ability to pass through the endothelial tight junctions of the nasal vascular bed (Chhajed, Sangale, & Barhate, 2011). Interestingly, the microgels of the 150 nm size displayed a lower striatal tissue concentration than the 250 nm microgels and more importantly, the microgels >250 nm were able to pass through the BBB and were detected in all three brain regions examined. The prior literature has described particles >150 nm in size to be excluded from BBB transport at sizes of 150 nm specifically when solid nanoparticles like that of the more solid PEG-PLGA nanoparticles are administered *in vivo* (Gaumet *et al.*, 2008). While not wishing to be limited by theory, this is perhaps due to the soft, flexible and relatively uncharged

character of these microgels, allowing them to orient into an ellipsoidal shape to squeeze through the endothelial tight junctions under physiological blood pressure. This property may be more possible at sizes >150 nm due to the presence of a greater number of less dense phase-separated polymer chains to the exterior of the microgel complex that would be more able to compress into an ellipsoid shape. All of the major organ tissue concentrations of 100 nm rhodamine-labelled microgels were shown to be higher than that of the other two microgel sizes, where the highest concentrations were found to be in organs involved in the detoxification and purification of blood (i.e. liver, kidneys and spleen) (Chhajed, Sangale, & Barhate, 2011). This is similar to major organ biodistributions seen when 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indotricarbocyanine Iodide (DiR)-loaded PEG-PLGA emulsions were administered to naive rats (Chen *et al.*, 2011). Due to the slow degrading nature of the microgels, the rhodamine-microgel signals observed in the tissue extracted should be that of the rhodamine-conjugated microgels and not free floating rhodamine. The observed brain tissue concentrations are very promising in that they prove that POEGMA microgels can effectively pass the BBB at relatively high concentrations.

[00134] With the initial proof that the POEGMA microgels were capable of crossing the BBB to be detected in tissue, the next step was to try a pilot study in which PAOPA-loaded microgels were administered to naïve rats using the IP route of administration. This initial pilot also showed that the use of POEGMA microgels may lead to a greater amount of PAOPA crossing the BBB to reach the brain. There were significant differences in POAPA concentration in the PFC perhaps due to the presence of the D2 receptor (though this is a lower D2 receptor density than the striatum) and the D4 receptor, in which both have been shown to bind PAOPA at the receptors allosteric site (Basu *et al.*, 2013; Verma *et al.*, 2005). In the initial trials, the HPLC results demonstrated that PAOPA reaches the striatum, which contains a high density of its allosteric D2 receptor target and in greater PAOPA tissue concentrations when a POEGMA microgel carrier is used (Weiner & Brann, 1989). Interestingly, there was no significant difference in the use of a drug carrier with respect to cerebellum tissue concentrations, which may potentially change with increasing duration of time or may be due lower binding affinity of PAOPA to the D2S over that of the D2L, which has only been shown to be expressed several times within the cerebellum and is subject to some debate (Ikai *et al.*, 1992). This aside, the present

results demonstrate promise in the use of POEGMA microgel drug carriers in the transport of PAOPA, and other APDs, through the BBB for the treatment of a variety of CNS disorders (one of these specifically being schizophrenia).

[00135]

[00136] While the present application has been described with reference to examples, it is to be understood that the scope of the claims should not be limited by the embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

[00137] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

TABLE 1: Microgel size, polydispersity index and zeta potential for each of the microgel samples

Microgel Type and Drug Encapsulated	**Microgel Size (nm)**	**Polydispersity Index (PDI)**	**Zeta Potential (mV)**
Empty Microgels	130±2	0.243±0.003	-13.6±1.0
Empty STL-Functionalized Microgels	122±2	0.132±0.005	-16.8±0.7
PAOPA-Loaded Microgels	142±1	0.132±0.008	-14.4±1.9
STL-Functionalized PAOPA-Loaded Microgels	114±1	0.156±0.061	-16.9±0.9

TABLE 2: Comparison of microgel concentration, encapsulation efficiency, drug loading capacity and cumulative release *in vitro*

Microgel Type and Drug Encapsulated	**Microgel Concentration (particles/mL)**	**Encapsulation Efficiency (%)**	**Drug Loading Capacity (%)**
Empty Microgels	3.47×10^{12}	-	-
Empty STL-Microgels	3.30×10^{12}	-	-
PAOPA-Loaded Microgels	3.57×10^{12}	$33.6 \pm 2.4\%$	$0.39 \pm 0.03\%$
STL-Functionalized PAOPA-Loaded Microgels	3.60×10^{12}	$30.2 \pm 1.9\%$	$0.35 \pm 0.03\%$

TABLE 3: Rhodamine-labeled microgel size, polydispersity index and microgel concentration

Microgel Type and Drug Encapsulated	**Microgel Size (nm)**	**Polydispersity Index (PDI)**	**Microgel Concentration (particles/mL)**
Rhodamine labeled Microgel 1	78.8±1.59	0.04±0.001	2.78×10^{11}
Rhodamine labeled Microgel 2	122.4±2.47	0.026±0.001	3.82×10^{11}
Rhodamine labeled Microgel 3	219.9±4.44	0.105±0.002	3.66×10^{11}

FULL CITATIONS FOR DOCUMENTS REFERRED TO IN THE APPLICATION

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Claims:

1. A microgel composition comprising:

a) a chemically cross-linked microgel having a particle size of about 20 nm to about 300 nm,; and

b) one or more substances for delivery to the brain,

wherein the substance is entrapped in and/or on the chemically cross-linked microgel.

2. The microgel composition of claim 1, wherein the chemically cross-linked microgels comprise a polymer that is cross-linked with a linker group that hydrolytically or enzymatically degrades under physiological conditions.

3. The microgel composition of claim 1 or 2, wherein the chemically cross-linked microgel comprises polymeric units comprising oligo(ethylene glycol) acrylate (OEGA), oligo(ethylene glycol) methacrylate (OEGMA), oligo(ethylene glycol) diacrylate (OEGDA), N-alkylacrylamides, diethylene glycol (meth)acrylate ($M(EO)_2MA$) or vinyl caprolactone (VCL), or copolymers thereof.

4. The microgel composition of claim 1 or 2, wherein the chemically cross-linked microgel comprises polymeric units comprising poly(ethylene glycol), poly(N-vinylpyrrolidone), poly(hydroxyethylmethacrylate) or carbohydrates.

5. The microgel composition of claim 3 or 4, wherein the polymeric units further comprise one or more co-monomer units selected from methacrylates, acrylates, acrylamides and vinyls.

6. The microgel composition of claim 5, wherein the polymeric units further comprise one or more co-monomer units selected from acrylic acid (AA), fumaric acid (FA), vinyl acetic acid (VAA), methacrylic acid (MAA), butylmethylacrylate (nBuMA), dimethylaminoethyl acrylate (DMAEA), acetoacetoxymethacrylate, acrylamide (AAm), aminoethyl methacrylate (AEMA), dimethylaminoethyl (meth)acrylate (DMAEMA) and N-(3-aminopropyl)methacrylamide (APMA)

7. The microgel composition of any one of claims 2 to 6, wherein the linker group that hydrolytically or enzymatically degrades under physiological conditions

comprises an acetal, activated ester, amide, anhydride, disulfide, hydrazide, and/or imine moiety.

8. The microgel composition of claim 7, wherein the linker group that hydrolytically or enzymatically degrades under physiological conditions comprises a disulfide moiety.

9. The microgel composition of claim 1, wherein the chemically cross-linked microgels comprise, or consist essentially of POEGMA cross-linked with a linker group comprising a disulfide.

10. The microgel composition of any one of claims 1 to 9, wherein the chemically cross-linked microgels are surface-functionalized with one or more molecules for cell targeting and/or internalization.

11. The microgel composition of claim 10, wherein the one or more molecules for cell targeting and/or internalization are selected from cell adhesive ligands lectins, cadherins, integrins, glycoproteins, mucoadhesive ligands, antibodies and aptamers

12. The microgel composition of claim 10, wherein the one or more molecules for cell targeting targets the microgel to cells in the nasal cavity.

13. The microgel composition of claim 12, wherein the one or more molecules for cell targeting is *Solanum tuberosum* lectin (STL).

14. The microgel composition of any one of claims 1 to 13, wherein the one or more substances for delivery to the brain are selected from substances that modulate one or more biological targets in the brain.

15. The microgel composition of claim 13, wherein the substances that modulate one or more biological targets in the brain are antipsychotic drugs.

16. The microgel composition claim 15, wherein the antipsychotic drug is selected from one or more of amantadine, amisulpride, apomorphine, aripiprazole, asenapine, blonanserin, bromocriptine, carbidopa, chlorpromazine, clotiapine, clozapine, divalproex, droperidol, entacapone, fluphenazine, haloperidol, iloperidone, levodopa, levomepromazine, lithium, loxapine, lurasidone, mesoridazine, molindone,

mosapramine, olanzapine, PAOPA, paliperidone, perospirone, perphenazine, pramipexole, prochlorperazine, quetiapine, remoxipride, risperidone, ropinirole, selegiline, sertindole, sulpiride, thioridazine, thiothixene, tolcapone, trifluoperazine, valproic acid, ziprasidone, zotepine and zuclopenthixol, or analogs or derivatives thereof.

17. The microgel composition of claim 16, wherein the antipsychotic drug is PAOPA or a PAOPA analog or derivative.

18. The microgel composition of claim 1, comprising (a) a water-swollen POEGMA polymer network cross-linked via disulfide bonds; and (b) PAOPA.

19. The microgel composition of any one of claims 1 to 13, wherein the one or more substances for delivery to the brain are selected from one or more substances that image one or more areas of the brain.

20. The microgel composition of claim 19, wherein the one or more substances that image one or more areas of the brain are selected from MRI imaging agents, fluorescent markers and radioactive imaging agents

21. A pharmaceutical or diagnostic comprising one or more of the microgel compositions of any one of claims 1 to 20 and a pharmaceutically acceptable carrier, diluent and/or adjuvant.

22. A method of treating a condition, disease and/or disorder that benefits from a modulation of targets in the brain comprising administering, to a subject in need thereof, an effective amount of one or more of the microgel compositions of any one of claims 1 to 18 wherein the one or more microgel compositions comprise one or more substances that modulate one or more biological targets in the brain.

23. A method of imaging the brain comprising administering, to a subject in need thereof, an effective amount of one or more of the microgel compositions of any one of claims 1 to 13 and 18-19, wherein the one or more microgel compositions comprise one or more substances that image one or more areas of the brain, and performing an imaging method on the subject

Figure 1

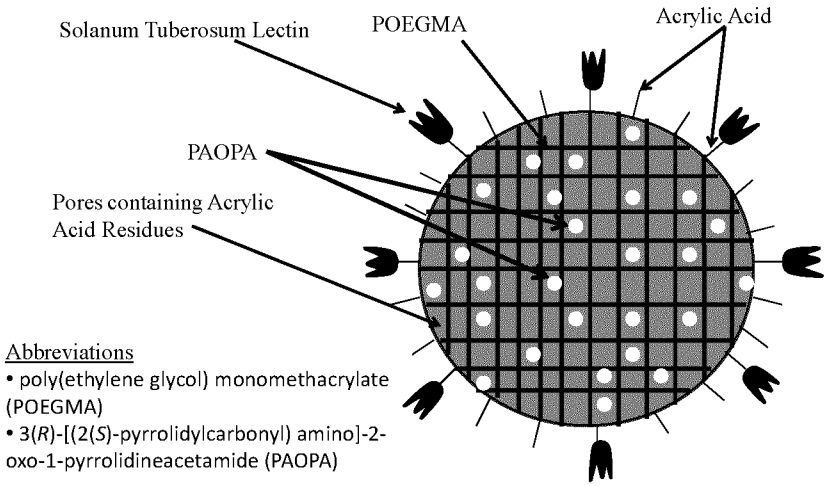

Solanum Tuberosum Lectin
POEGMA
Acrylic Acid
PAOPA
Pores containing Acrylic Acid Residues
Abbreviations
• poly(ethylene glycol) monomethacrylate (POEGMA)
• 3(R)-[(2(S)-pyrrolidylcarbonyl) amino]-2-oxo-1-pyrrolidineacetamide (PAOPA)

Figure 2

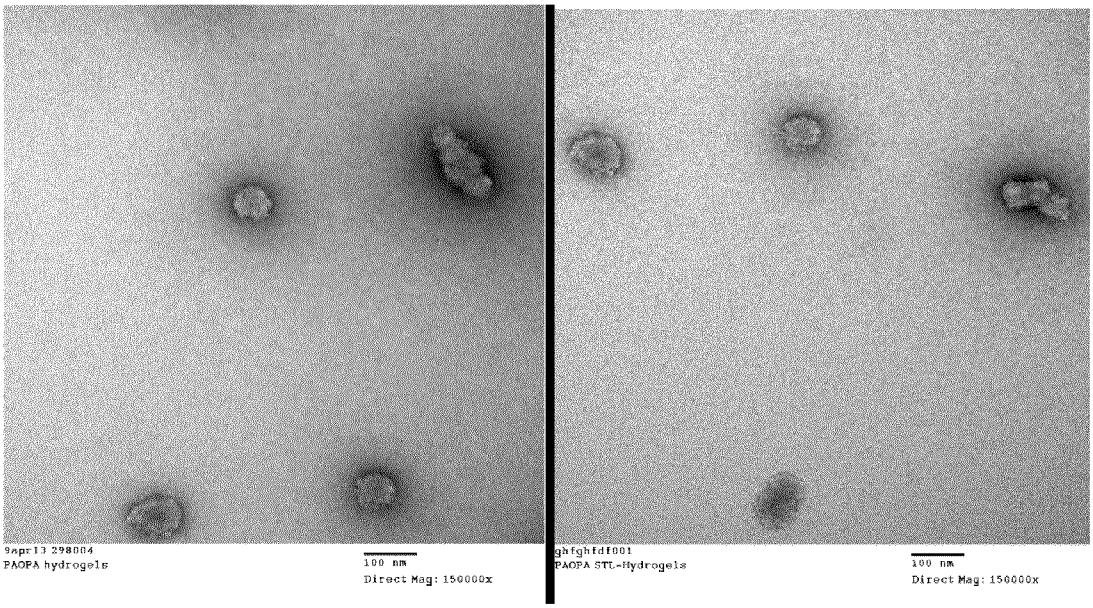

9apr13 298004
PAOPA hydrogels
100 nm
Direct Mag: 150000x
ghfghfdf001
PAOPA STL-Hydrogels
100 nm
Direct Mag: 150000x

Figure 3

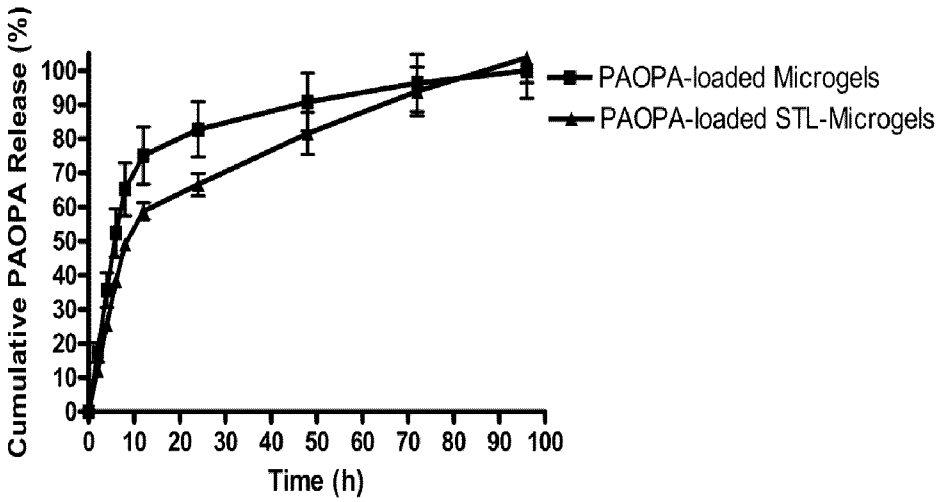

Cumulative PAOPA Release (%)
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100
Time (h)
PAOPA-loaded Microgels
PAOPA-loaded STL-Microgels

Figure 4

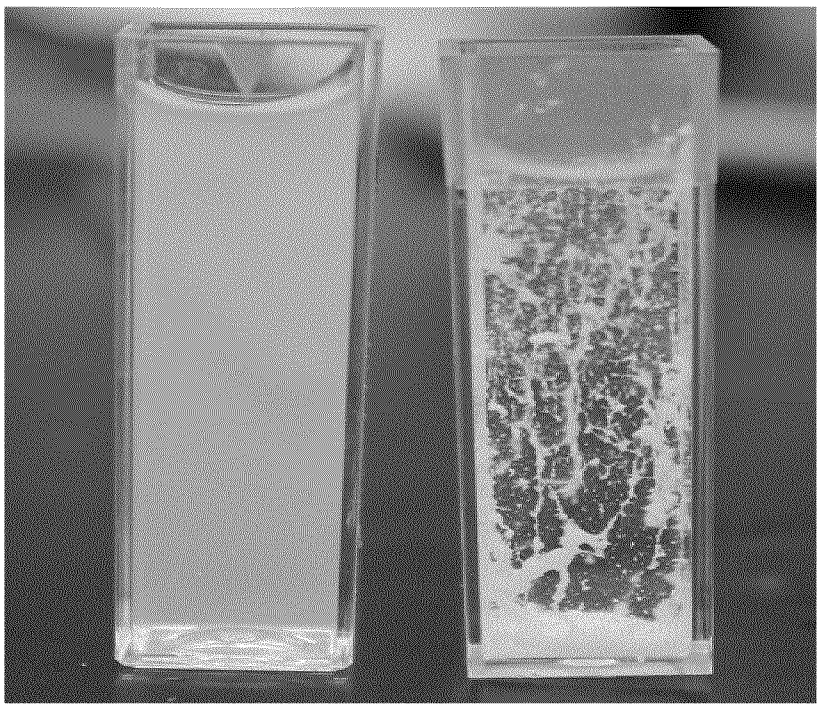


Figure 5

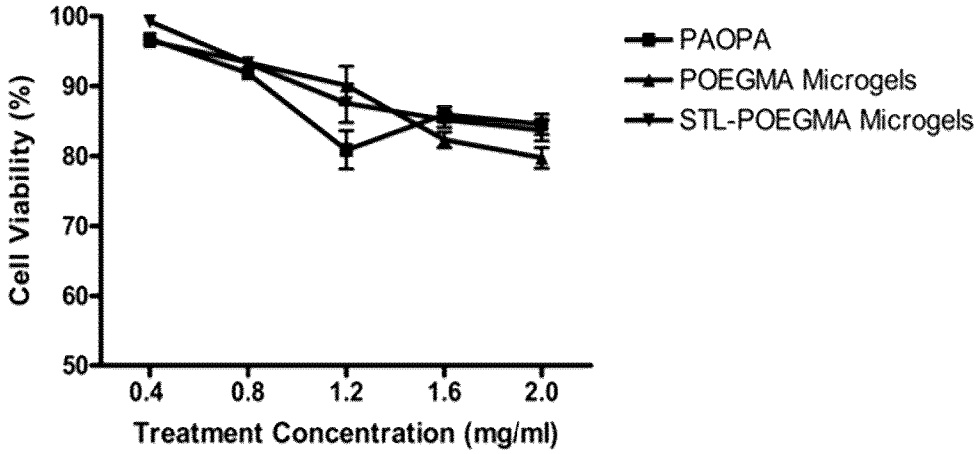

100
90
80
70
60
50
Cell Viability (%)
0.4
0.8
1.2
1.6
2.0
Treatment Concentration (mg/ml)
PAOPA
POEGMA Microgels
STL-POEGMA Microgels

Figure 6

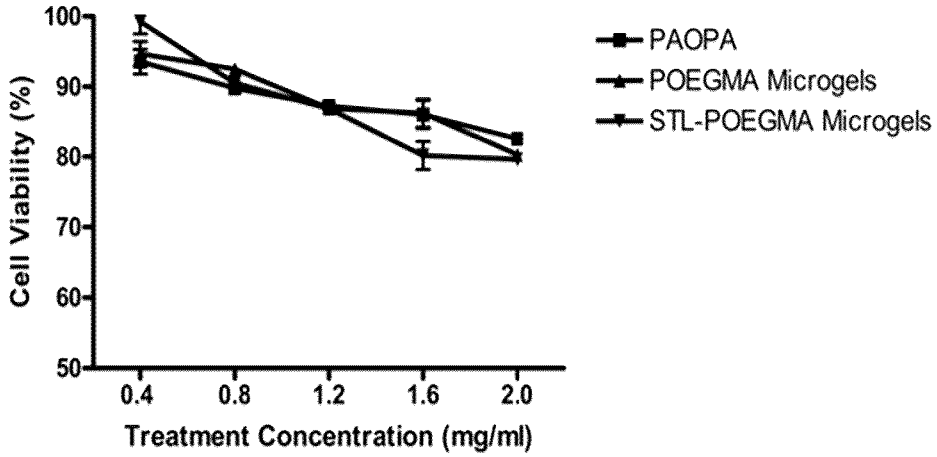

100
90
80
70
60
50
Cell Viability (%)
0.4
0.8
1.2
1.6
2.0
Treatment Concentration (mg/ml)
PAOPA
POEGMA Microgels
STL-POEGMA Microgels

Figure 7

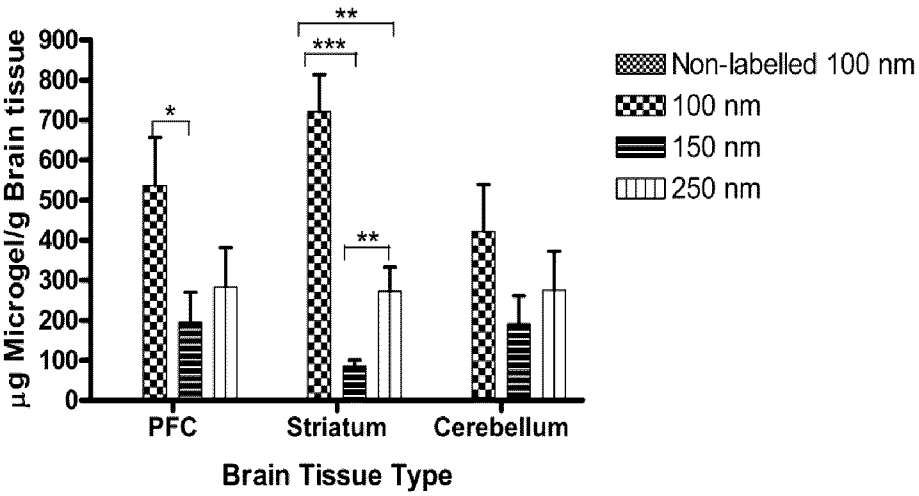

µg Microgel/g Brain tissue
900
800
700
600
500
400
300
200
100
0
*
**
***
**
PFC
Striatum
Cerebellum
Brain Tissue Type
Non-labelled 100 nm
100 nm
150 nm
250 nm

Figure 8

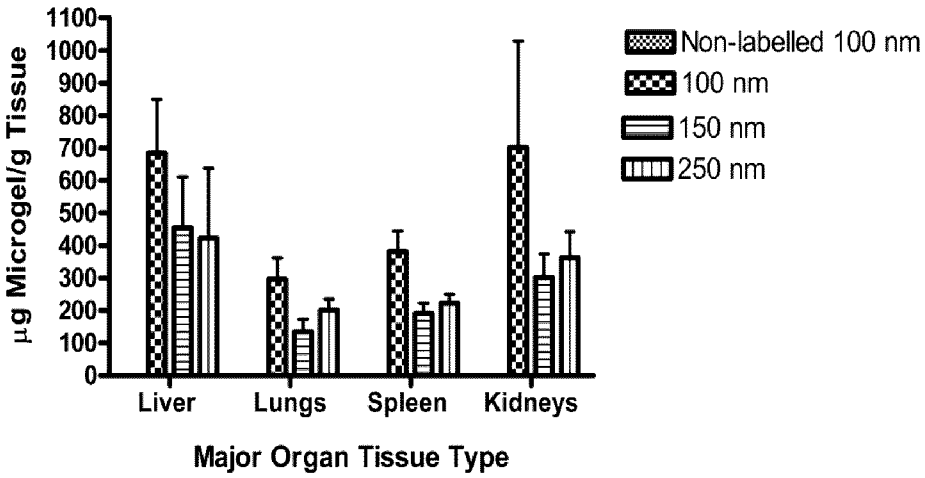
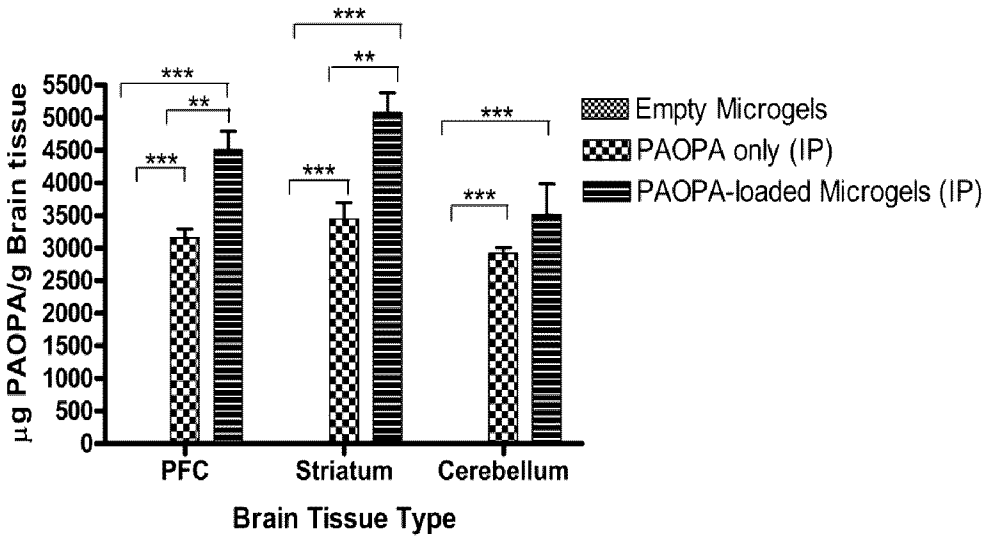

µg Microgel/g Tissue
1100
1000
900
800
700
600
500
400
300
200
100
0
Liver
Lungs
Spleen
Kidneys
Major Organ Tissue Type
Non-labelled 100 nm
100 nm
150 nm
250 nm

Figure 9


µg PAOPA/g Brain tissue
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
***
**
***
***
**
***
***
***
PFC
Striatum
Cerebellum
Brain Tissue Type
Empty Microgels
PAOPA only (IP)
PAOPA-loaded Microgels (IP)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2014/050827

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 9/10*** (2006.01), ***A61K 38/05*** (2006.01), ***A61K 47/34*** (2006.01), ***A61K 47/42*** (2006.01), ***A61K 49/00*** (2006.01), ***A61P 25/18*** (2006.01) (more IPCs on the last page)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 9/10, A61K 38/05, A61K 47/34, A61K 47/42, A61K 49/00, A61P 25/18, C08J 3/075, C08J 3/24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
SCOPUS, TOTALPATENT (including WO, US, EP, JP, and CA databases). Search terms: brain, microgel, nanogel, gel, nasal, lectin, PAOPA, Pyrrolidineacetamide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0180964 A1 (PAPINENI ET AL) 16 JULY 2009 (16-07-2009)	1-7, 10-12, 14, 15, 19-23
Y	CA 2 830 052 A1 (KANNAN, R. ET AL) 06 OCTOBER 2011 (06-10-2011)	8-9, 18
Y	US 8 394 391 B2 (BAE ET AL) 12 MARCH 2013 (12-03-2013)	13
Y	WO 2012/027825 A1 (JOHNSON, ET AL) 08 MARCH 2012 (08-03-2012)	16-17

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2014 (27-11-2014)	22 December 2014 (22-12-2014)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	ADAM MACKENZIE (819) 994-6514

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 22
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 22 is directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under Rule 39.1(iv) of the PCT. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claim 22.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant=s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2830052A1	06 October 2011 (06-10-2011)	CA2830052A1	06 October 2011 (06-10-2011)
		EP2552458A1	06 February 2013 (06-02-2013)
		EP2552458A4	27 August 2014 (27-08-2014)
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US2009180964A1	16 July 2009 (16-07-2009)	US2009180964A1	16 July 2009 (16-07-2009)
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WO2012027825A1	08 March 2012 (08-03-2012)	None	
CA2627903A1	28 September 2009 (28-09-2009)	None	

C08J 3/075 (2006.01) , ***C08J 3/24*** (2006.01)