NOVEL ANTIPSYCHOTIC DRUG CARRIERS

## NOVEL ANTIPSYCHOTIC DRUG CARRIERS: THE DEVELOPMENT OF NANOPARTICLE AND MICROGEL DRUG CARRIERS FOR ANTIPSYCHOTIC DELIVERY IN THE TREATMENT OF SCHIZOPHRENIA

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Science

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## **DESCRIPTIVE NOTE**

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McMaster University MASTER OF SCIENCE (2013) Hamilton, Ontario (Neuroscience) TITLE: Novel Antipsychotic Drug Carriers: The Development of Nanoparticle and Microgel Drug Carriers for Antipsychotic Delivery in the Treatment of Schizophrenia AUTHOR: Justin E. Piazza, H.B.Sc. (McMaster University) SUPERVISOR: Dr. Ram K. Mishra NUMBER OF PAGES: xx, 136

## ABSTRACT

Lectin-functionalized, Poly [oligo(ethylene glycol) methyl ether methacrylate] (POEGMA) loaded with 3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-2-oxo-1pyrrolidineacetamide (PAOPA) and poly(ethylene glycol)-block-poly(D,L-lactic-coglycolic acid) (PEG-PLGA) nanoparticles loaded with haloperidol were prepared with narrow size distributions and sizes < 135 nm. The microgels and nanoparticles exhibited high Solanum tuberosum lectin (STL) conjugation efficiencies, encapsulation efficiencies, and drug loading capacities. The *in vitro* release of PAOPA and haloperidol was slow in physiological conditions over 96 hours, demonstrating minimal drug leakage and the potential for efficient drug transport to the targeted brain tissue. POAPA, POEGMA and the STL-functionalized POEGMA microgels were found to be non-toxic in both cell lines, indicating that they would not be toxic when administered intranasally or when they reach the brain. The nasal epithelial cell uptake of rhodamine-labelled microgels was higher in cells when the STL-functionalization was present. All haloperidol-loaded nanoparticle formulations were found to be highly effective at inducing catalepsy, while intranasal administration of STL-functionalized nanoparticles using the intranasal spray device increased the brain tissue haloperidol concentrations by 2-3.5 fold compared to STL-functionalized particles administered intranasally with a pipette. For the first time, brain tissue concentrations of rhodamine-labelled microgels confirmed that microgels are capable of passing the blood-brain barrier and that this uptake is size dependent. These formulations demonstrate promise in the reduction of the drug dose necessary to produce

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a therapeutic effect with antipsychotic drugs for the treatment of schizophrenia using a non-invasive route of administration.

## ACKNOWLEDGEMENTS

The experience and mentoring that I have had during my time as a graduate student working under Dr. Ram K. Mishra have been invaluable. There is no way that I believe I can ever truly thank everyone that has been there for me over the past two years, though I do want to make specific note of a few:

I would first like to express my thanks to my supervisor, Dr. Ram Mishra, as well as my supervisory committee members, Dr. Todd Hoare, Dr. Bhagwati Gupta and Dr. Benito Frey. To Dr. Mishra: You gave me the opportunity to pursue so much and to broaden my horizons in fields that I never dreamed I would have the chance to dive into (ex. chemical engineering, pneumatic development, intellectual property law). Your constant encouragement, patience and close attention to detail have been paramount to my success as a graduate student, providing an excellent example of what a truly great supervisor should be. As a strongly motivated and eccentric first year graduate student, I wasn't sure what to quite expect when coming to McMaster University. At the time, all I knew was that I wanted to do everything I could to contribute to the knowledge we have on mental health treatment. I was completely unaware of how this opportunity and these experiences under your supervision would solidify my dream to pursue mental health research as an MD/MSc. To Dr. Hoare: I am incredibly grateful for your leadership and advice. You were always willing to meet to discuss any issues that I had throughout my project and I will always remain in awe of the large knowledge base you have across so many disciplines. This dedication and cross-disciplinary approach are what I wish to employ one day if given the opportunity to pursue research as a supervisor at an accredited university. To Dr. Gupta: I truly appreciate your attention to fine detail in the composition of any study and your ability to point out possible confounds or future issues that may result in any experiment. This type of scientific inquiry is what ensures the absolute validity of any study performed and solidifies the significance of all results obtained. To Dr. Frey: I truly appreciate your dedicated support of not only my academic goals, but that of my clinical aspirations. Your advice on the road to becoming a physician/researcher has been invaluable. I am still in awe of your ability to simultaneously perform research, clinical work and run the Neuroscience program in conjunction with Dr. Murphy.

I would also like to thank all of my colleagues within the Mishra lab for all of their help and advice over the years: Ritesh Daya, Mattea Tan, Christal Sookram, Dipa Basu, Luke Molinaro, Asim Siddiqi, Kristen Terpstra, Jayant Bhandari and everyone else in the lab. You have been an outstanding group of people to not only work with, but to have gotten to know and I wish you all the best. To Nancy Thomas: you are easily the best lab technician of all time, through your uncanny ability to troubleshoot, extensive knowledge base, invaluable advice and how you're always willing to help any of us out any time we're in need.

As well, I want to thank everyone in the Neuroscience program for making this experience unforgettable from our time in NS 700 through to the Society for Neuroscience conference. Lastly, I am eternally grateful to my family for their unwavering support of the pursuit of my aspirations and hope to make them proud.

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## LIST OF ABBREVIATIONS AND SYMBOLS

- 3-MT 3-methoxytyramine
- 6-OHDA 6-hydroxydopamine
- AADC L-amino acid decarboxylase
- $\alpha$ - $\beta$ -GP  $\alpha$ - $\beta$ -glycerophosphate
- ACD acid-citrate-dextrose
- ANOVA Analysis of Variance
- APD antipsychotic drug
- BBB Blood brain barrier
- BCA bicinchoninic acid
- BINA biphenyl-indanone A
- BSA bovine serum albumin
- CaM calmodulin
- cAMP cyclic adenosine monophosphate (cAMP)
- CDPPB 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide
- CNS central nervous system
- COMT catechol O-methyltransferase
- CPPHA N-{4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide
- CSF cerebral spinal fluid
- D2<sup>High</sup> high affinity state of the D2 receptor
- D2L dopamine receptor long splice variant
- D2<sup>Low</sup> the low-affinity state of the D2 receptor

- D2S dopamine receptor short splice variant
- DAT dopamine transporter
- DCM dichloromethane
- DFB 3,3'-Difluorobenzaldazine
- DiR 1'-dioctadecyl-3,3,3',3'-tetramethyl indotricarbocyanine Iodide
- DLC drug loading capacity
- DMSO dimethyl sulfoxide
- DOPAT 3,4-9 dihydroxyphenylacetic acid
- DTT dithiothreitol
- EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
- EDTA Ethylenediaminetetraacetic acid
- EE encapsulation efficiency
- EGDMA Ethylene glycol dimethylacrylate
- GABA γ-Aminobutyric acid
- GPCR G-protein coupled receptors
- HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
- <sup>1</sup>H NMR proton nuclear magnetic resonance
- hPEA-mGel multi-responsive hyperbranched poly(ether amine) microgels
- HPLC high performance liquid chromatography
- HTCC N-[(2-hydroxy-3-trimethylammonium) propyl] chitosanchloride
- IN intranasal
- IP intraperitoneal

- KCl potassium chloride
- KPS potassium persulfate

L-DOPA - L-3,4-dihydroxyphenylalanine

LCST - lower critical solution temperature

*n*BuMA - *n*-butyl methacrylate

Mal - maleimide

- MalPEG maleimide polyethylene glycol
- MAO monoamine oxidase

Me-Methoxy

- MePEG methoxy polyethylene glycol
- MES 2-(N-morpholino)ethanesulfonic acid
- mGluR2 metabotrobic glutamate receptor subtype 2
- mGluR5 metabotrobic glutamate receptor subtype 5
- MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
- MTT 3-(4,5 dimethylthiozol-2-yl)-2,5-diphenyl-tetrazolium bromide
- NPA [<sup>3</sup>H] N-propylnorapomorphine
- NTA nanoparticle tracking analysis
- PAOPA 3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide
- PBS phosphate buffered saline
- PDI polydispersity index

PEG-PLGA - Poly(ethylene glycol)-block-poly(D,L-lactic-co-glycolic acid)

PFA - paraformaldehyde

- PFC prefrontal cortex
- PKA protein kinase A
- PLG L-prolyl-L-leucyl-glycinamide
- PMSF phenylmethylsulfonyl fluoride
- PNIPAM-PMAA poly(N-isopropylacrylamide)-poly(methacrylic acid)
- POEGMA Poly [oligo(ethylene glycol) methyl ether methacrylate]
- PVA Polyvinyl alcohol
- PVA-g-AAm PVA-grafted-acrylamide
- P2Y2 P2U G-protein-coupled receptor
- STL Solanum tuberosum lectin
- SLN solid lipid nanoparticles
- TH tyrosine hydroxylase
- TEM transmission electron microscope
- VMAT vesicular monoamine transporter
- VTA ventral tegmental area

## I) SUMMARY OF ACCOMPLISHMENTS

## **Publications Submitted:**

- "Intranasally Administered Lectin Functionalized PEG-PLGA Nanoparticles for the Treatment of Schizophrenia" to submitted to the journal Nanomedicine: Nanotechnology, Biology and Medicine (IF = 6.692)
- This publication is currently under editorial review

#### Grants Submitted/Participated In:

- "The Development of a Multi-dose Intranasal Spray device for studying delivery mechanisms and Neural Bioavailability following the Nasal Inhalation" submitted to NSERC for the **Alexander Graham Bell Canada Graduate Scholarship** though unsuccessful

#### Presentations and Conferences Attended:

- A poster presentation was given at the Southern Ontario Neuroscience Association (SONA) conference in April of 2012 held in Toronto, ON
- A poster presentation was given at the Faculty of Health Sciences Research Plenary in May of 2012 held in Hamilton, ON
- A poster presentation was given at the Society for Neuroscience (SFN) conference in October of 2012 held in New Orleans, LA
- A poster presentation was given at the Department of Psychiatry and Behavioral Neurosciences' Psychiatry Research Day in April of 2013 held in Hamilton, ON
- A poster presentation was given at the McMaster Industry Liaison Office (MILO) Innovation Showcase in May of 2013 held in Hamilton, ON
- A poster presentation was given at the Faculty of Health Sciences Research Plenary in May of 2013 held in New Orleans, LA

#### Awards:

 The Neuroscience (MiNDs) program provided a \$350 travel award for the purposes of attending the SFN conference in October of 2012 held in New Orleans, LA

- The Department of Psychiatry and Behavioral Neurosciences provided a \$300 poster competition award for winning the Graduate Basic Research Competition at Psychiatry Research Day in May of 2013 held in Hamilton, ON
- The McMaster Industry Liaison Office (MILO) provided a \$1000 poster competition award for winning the Most Groundbreaking Research Award at the MILO Innovation Showcase in May of 2013 held in Hamilton, ON

#### Major Techniques/Skills Attained:

 I have gained the necessary skills to use: Zeta Potential Analysis (ZPA), Dynamic Light Scattering (DLS), Confocal microscopy, high performance liquid chromatography (HPLC), gel permeation chromatography (GPC), Amine Derivatizations, EDC Reactions, Michael Addition Reactions, Ring Opening Polymerizations, Free Radical Polymerizations, Receptor Binding Assays, drug tissue extractions, bicinchoninic acid (BCA) assays, Schizophrenia behavioral models

## New Collaborations:

- I have been actively collaborating with Dr. Hoare and his post-doc, Dr. Neils Smeets from chemical engineering in the production of haloperidol-loaded nanoparticles, which led to the manuscript we recently submitted. We have also been working on using microgel drug carriers for PAOPA (see below)
- I have recently also been collaborating with Dr. Jay Schneider from Thomas Jefferson University through a chance meeting during my poster presentation at SFN this year

## **1 INTRODUCTION**

Schizophrenia still remains one of the most debilitating of all the neuropsychiatric illnesses, leaving an unfortunately large burden upon the shoulders of patients and those that support them (ex. family, friends, spouses, etc.). The prognosis for long-term pharmacological success in schizophrenia also remains quite poor and many of the pharmacotherapies used in treating schizophrenia are riddled with life-altering side effects. On top of this, the majority of the drugs entering the body never reach their molecular targets within the brain and remain in systemic circulation until metabolism, resulting in many of the aforementioned side effects. This makes it imperative to develop antipsychotic drug (APD)-loaded drug carriers capable of effective transport across the blood-brain barrier (BBB) carrying safer antipsychotics that still remain functional following release from their drug carriers. Therefore, the goal of the present study was to demonstrate the *in vivo* efficacy of APD-loaded drug carriers designed for cell-targeting specific to the intranasal route of administration using a novel intranasal spray device in rodents.

#### 1.1 Schizophrenia

Schizophrenia has been described as one of the most incapacitating across all mental illness diagnoses, affecting 1% of the world's population and leading to significant annual financial costs on health care system budgets globally (Nicholl *et al.*, 2010; Sawa & Snyder, 2002). The direct healthcare and non-healthcare costs of schizophrenia create a substantial economic burden at approximately \$2.02 billion in 2004 (Goeree *et al.*, 2005). Outside of the direct costs of schizophrenia, the indirect costs

regularly surpass that of the direct costs annually at a staggering additional \$4.83 billion. The indirect costs include increased unemployment, a loss of workplace productivity, caregiver burden, and increased mortality rates resulting from suicide (Nicholl *et al.*, 2010).

With this in mind and the fact that there is no known cure for schizophrenia, it is imperative that a new as well as more effective form of treatment are developed in order to treat the pathophysiologies of this debilitating illness.

#### 1.1.1 Epidemiology

Though schizophrenia has been found to be equally prevalent in both men and women, men typically present a significantly earlier age of onset (24.89 years) over that of women (29.01 years) and a greater severity of symptoms (Segarra *et al.*, 2012). A study out of Quebec recently combined primary health care and mental health data showing high incidence rates of 42 per 100,000 if only hospital admissions for schizophrenia were counted and a shocking 94 per 100,000 when all physician contacts were counted (Vanasse *et al.*, 2012). Saha *et al.*, 2005 described how the incidence rates of schizophrenia globally demonstrate great variance, yet their systematic review of 1,721 prevalence estimates from 188 studies in 46 different countries revealed an incidence rate of 15.2 per 100 000 population and a prevalence rate of 7.2 per 1000 population.

#### 1.1.2 Symptomatology

In order to effectively understand how to treat schizophrenia, it is important to understand the symptoms and the underlying physiological mechanisms that create them. The clinical manifestations of schizophrenia are characterized by three divisions of symptomatology: positive symptoms, negative symptoms, and cognitive dysfunctions or deficits (Crow, 1980; van Os & Kapur, 2009).

The positive symptoms represent the presence of behaviours composed of excesses or sensory distortions in patients with the disease including paranoia, hallucinations, and delusions, which are absent within the healthy population (Crow, 1980). Hallucinations experienced by patients are generally auditory in nature, where the patient may hear or converse with voices. Paranoid delusions are also frequently observed and may include unrealistic beliefs of persecution, somatics (i.e. functionality or structure) or grandeur (Schultz, North, & Shields, 2007). The negative symptoms are conversely present in the healthy population, but are absent within individuals diagnosed with schizophrenia. These negative symptoms may include avolition (a lack of drive or motivation), a lack of apathy, anhedonia (inability to experience pleasure), and social isolation (Crow, 1980).

The cognitive deficits commonly identified in schizophrenic patients include dysfunctions of memory (verbal, visual and working), attention, reasoning/problem solving, linguistic capabilities and executive functioning or decision making processes (Dickinson *et al.*, 2004; Nuechterlein *et al.*, 2004). There are two additional documented schizophrenia subtypes that have been noted in patients diagnosed with schizophrenia. The first being that of catatonia or a motor immobility or rigidity, where a patient may also ignore all external stimuli and the second being inappropriate affect, where the

patient may display a highly inappropriate emotional response in certain contexts (ex. laughing uncontrollably at a funeral) (Peralta, de Leon, & Cuesta, 1992).

#### **1.2 Pathophysiology**

#### 1.2.1 Dopamine

Dopamine is part of the catecholamine neurotransmitter family that selectively binds to and activates any of the five classes of dopamine receptors (D1-D5) (Figure 1). This organic molecule is produced via dopaminergic neurons within the brain and the adrenal gland. Dopamine may also serve as a precursor to the production of other catecholamines through a series of chemical reactions yielding norepinephrine and epinephrine (Hornykiewicz, 1966).

Within the brain, dopamine signalling has been shown to modulate the expedited actions of the neurotransmitters glutamate and  $\gamma$ -Aminobutyric acid (GABA) to influence reward-motivated behavior, memory, motor function, emotion, and executive functioning (Berridge, 2007; Chéramy *et al.*, 1986). While outside of the nervous system, dopamine is involved in vasodilation to control blood pressure, the senses, sodium excretion within the kidneys and insulin production within the liver (Contreras *et al.*, 2000).

At neuron terminals, dopamine is synthesized from either the essential amino acid precursor, phenylalanine, obtained through diet or the non-essential amino acid tyrosine. Through the action of tyrosine hydroxylase (TH), tyrosine is first converted to L-3,4dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (TH) during this ratelimiting step in dopamine synthesis (Contreras *et al.*, 2000). Next, L-amino acid decarboxylase (AADC) converts L-DOPA into dopamine and dopaminergic neurons then package dopamine into synaptic vesicles using vesicular monoamine transporter (VMAT) for release into the synapse (Miyake *et al.*, 2011).

If the neurons or cells use the norepinephrine neurotransmitter instead, dopamine is converted into norepinphrine via enzyme dopamine  $\beta$  hydroxylase (Contreras *et al.*, 2000). The norepinephrine molecule can also be further processed into epinephrine via the action of phenylethanolamine N-methyltransferase (Gordon *et al.*, 1966). Excess dopamine within the synapse can be reuptaken by the dopamine transporter (DAT), where it may be metabolized into homovanillic acid, with the intermediates 3,4-9 dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT) by monoamine oxidase (MAO) and catechol O-methyltransferase (COMT) (Velasco & Luchsinger, 1998).

Dopamine has been implicated in a number of central nervous system disorders including Parkinson's disease through dopaminergic neurodegeneration within the striatum, schizophrenia due to the dysregulation of dopamine signaling, dopamine transporter dysfunction in attention deficit hyperactive disorder (ADHD) and bipolar disorder due to hypomethylation of the MB-COMT promoter (Abdolmaleky *et al.*, 2006; Contreras *et al.*, 2000).



Figure 1: The chemical structure and synthesis of dopamine.

#### **1.2.2** The Dopamine Hypothesies

The longest enduring hypothesis within schizophrenia pathophysiology research is the dopamine hypothesis, which was formed in the 1950s with the finding that dopamine D2 receptor antagonists successfully treat the positive symptoms of schizophrenia (Stone et al. 2007). Schizophrenia has also been shown to be an ailment of dopamine excess in that dopamine synthesis is upregulated in drug-naive and prodromal schizophrenia patients (Hietala *et al.*, 1999; Howes *et al.*, 2009).

This meaning that the main component of this theory is that psychoses are a manifestation of dysregulated dopamine signaling as a result of a hyperdopaminergic state within the striatum and hypodopaminergic signaling within the prefrontal cortex (PFC) (Bennett, 1998; Tsai & Coyle, 2002). The mesocortical and mesolimbic dopaminergic pathways have been described as affecting the symptomatology seen within schizophrenic patients (Bennett, 1998). The mesolimbic dopaminergic pathway projects from the ventral tegmental area (VTA) into the limbic system via the nucleus accumbens, where pathway dysfunction may result in many of the positive symptoms of schizophrenia due to deficits in emotion and motivation (i.e. reward behaviors) (Bennett, 1998; Green *et al.*, 1999). This link between mesolimbic dopaminergic supersensitivity or hyperfunctionality and the increase in positive symptoms may also explain why treatment of these symptoms is possible with dopamine receptor antagonists has been so successful (Miyake *et al.*, 2011).

However, with the progression of research into the hyperdopaminergic theory, an extensive number of debates have led to the realization that more extensive circuitry is

involved (Howes & Kapur, 2009). Studies on cortical hypofunctionality have revealed that patients with schizophrenia also have associated deficits in dopaminergic neurotransmission within the PFC (Abi-Dargham & Moore 2003; Akil et al., 1999; Laruelle et al., 2003). The mesocortical dopaminergic pathway may be involved in executive function as mesocortical dopaminergic neurons project from the VTA to sites in the cortex. Dysfunctions resulting in hypoactivity in this pathway have not only been linked to the positive and negative symptoms, but some of the cognitive deficits in schizophrenia as well (Bennett, 1998). It has been described previously that PFC dysfunction (i.e. hypofrontality) and decreased prefrontal dopamine contribute to the negative and cognitive dysfunction observed in schizophrenic patients (Andreasen, 1999; Stone *et al.*, 2007). This is only a small subset of the incredibly extensive circuitry and pathways that may be involved in complex pathophysiology of schizophrenia (Carr & Sesack, 2000; Minzenberg et al., 2009; Thierry et al., 2000; Zahm & Heimer, 1990). With all of this in mind, it is important to note that region-specific drug activity may be necessary for adequate treatment of each of the three symptom divisions.

#### **1.3 Dopamine Receptors**

Dopamine receptors can be classified as G-protein coupled receptors (GPCRs) and can be found throughout the central nervous system as well as the periphery (Hornykiewicz, 1966). There are 5 known subtypes of the dopamine receptor split into two families of receptors, the D1-like and D2-like families of receptors (Neve, Seamans & Trantham-Davidson, 2004).

#### **1.3.1 The D1 receptor family**

The D1-like receptors (includes the D1 and D5 receptors) are completely postsynaptic and couple to  $G\alpha$  subunit to stimulate adenylyl cyclase, which leads to the upregulation of cyclic adenosine monophosphate (cAMP) production (Bouvier, 2001). The D1-like receptors are at greatest densities in regions involved in movement, cognition, and reward. Interestingly, D1 receptors show higher expression than the D5 receptor, perhaps due to its greater role in motivational behavior within the shell of the Nucleus accumbens (Schultz, North, & Shields, 2007). More specific to schizophrenia, altered DA receptor mRNA expression and decreased D1-like DA receptor expression have been observed in schizophrenia patients, which has been found to correlate with poor performance on the Wisconsin Card Sorting Task which tests frontal lobe function (Meador-Woodruff et al., 1997; Okubo et al., 1997; Stefanis et al., 1998). Prior research has demonstrated that increased D1 receptor antagonist binding potential and D1 receptor hypoactivity in the dorsolateral PFC were found to be a strong predictor of poor performance on working memory tasks as well in schizophrenic patients (Abi-Dargham et al., 2002; Abi-Dargham & Moore, 2003).

#### **1.3.2 The D2 receptor family**

D2-like receptors (include D2, D3, and D4 receptors) are localized on either the pre or postsynaptic neuronal membranes and couple with the Gαi/o subunit to inhibit adenylyl cyclase (Neve, Seamans, & Trantham-Davidson, 2004). Through Gαi/o subunit coupling, the D2 receptor can down-regulate cAMP expression, reducing the activation of cAMP-dependent protein kinase A (PKA) and in a more grandiose effect, altering the

permeability of potassium channels (Girault & Greengard, 2004). Additionally, The D2 receptor possesses two splice variants: the short (D2S) and long (D2L) receptor splice variants (Zhang *et al.*, 2007). The D2S and D3 receptors are generally classified as presynaptic autoreceptors in the mesocortical dopaminergic projections that play a modulatory role through feedback mechanisms in the synthesis, storage, and release of dopamine (Bertolino *et al.*, 2009). D3 receptor expression has thus far been shown to be localized within the striatum and nucleus accumbens, where the Ser9Gly polymorphism of the D3 gene has been linked to an enhanced vulnerability and more severe psychotic symptoms associated with schizophrenia (Reynolds *et al.*, 2005).

The D2S receptor has been shown to be primarily expressed via midbrain dopaminergic neurons, where it may inhibit DA synthesis via TH phosphorylation and activity via the modulation of the cAMP pathway, the ERK1/2 pathway, or both (Lindgren *et al.*, 2003). Therefore any dysfunction of the D2S could potentially lead to a decrease in midbrain GABA release probably through the binding of the G<sub>β</sub>i subunit to the N-type Ca<sup>2+</sup> channels  $\alpha$ 1 subunit, preventing presynaptic GABA release (Neve, Seamans, & Trantham-Davidson, 2004). This would in turn have a dramatic effect on dopaminergic neurons within the midbrain leading to disinhibition of the thalamus and result in positive symptoms such as delusions or hallucinations.

The D2L isoform mainly functions as a postsynaptic receptor, targeted by anatagonists such as haloperidol and works in tandem with the D1 receptor to influence several important processes (Bertolino *et al.*, 2009). The D2L isoform may potentially act as a negative modulator in enhancing DAT uptake due to significant DAT/D2L

coexpresion via D2-DAT interaction (Lee *et al.*, 2007). This may have several implications on hyperdopaminergic function within the striatum and positive symptom presentation in schizophrenic patients. This thesis will primarily focus on the effects of drug carrier delivered allosteric modulator on dopamine D2 receptors in relation to schizophrenia.

#### 1.4 Antipsychotic Drugs and Orthosteric Activation

In the absence of a "cure" for schizophrenia, there have been three generations of antipsychotic drugs (APDs) used to treat schizophrenic symptoms. Though there is a great deal of mechanistic and drug targeting variance among different APDs, most are dopamine D2 receptor antagonists competing with endogenous dopamine to reduce hyperdopaminergic activity within the striatum (Meltzer, 1991; Mukherjee *et al.*, 2001).

#### 1.4.1 Traditional ("Typical") Antipsychotics

The first generation or "typical" APDs are comprised of the phenzothiazines, butyrophenones, and thioxanthenes, (including chlorpromazine and haloperidol). These hydrophobic APDs exert their pharmacological effect via directly competing with endogenous dopamine in the antagonism of the dopamine D2 receptors within the mesolimbic and mesocortical pathways (Leucht *et al.*, 2009; Schultz, North, & Shields, 2007; Stone, Morrison, & Pilowsky, 2007). Typical APDs generally only show efficacy in treating the positive symptoms of schizophrenia and in relapse prevention, while presenting little to no improvement of patients' negative symptoms or cognitive dysfunction (Leucht *et al.*, 2009). Beyond this, patient adherence to typical APD

treatment is low due to somatic resistance, low responsiveness to treatment and the knowledge that long term-usage results in extrapyramidal side effects (i.e. movement disorders such as Tardive Dyskinesia) (Jeste *et al.*, 1999). This occurs through typical APD binding to the orthosteric site of the D2 receptor, which generally demonstrates a slow rate of dissociation (K<sub>d</sub>) from the receptor and in the long term leads to dopaminergic neuron excitotoxicity creating movement disorders (Kapur & Seeman, 2000; Sharma et al., 2003). This has also been confirmed via the production of vacuous chewing movements (model of Tardive Dyskinesia) in rodent models of schizophrenia after haloperidol treatment due to the excitotoxic effects of increased synaptic dopamine in the striatum (Sharma et al., 2003).

#### **1.4.2** The Second Generation ("Atypical Antipsychotics")

As a result of the typical APD treatment related extrapyramidal side effects and the absence of robust efficacy in treating the schizophrenic negative symptomatology or cognitive dysfunction with typical APDs, APD development pushed forward into the second generation of APDs ("Atypicals") in the early 1960s (Deutch *et al.*, 1991;Meltzer, 2004). This new generation of APDs was clinically distinct from traditional APDs by not only the lower rates of extrapyramidal side effects, but their ability to treat negative symptoms of schizophrenia as well (Leucht *et al.*, 2009; Meltzer, 2004; Stahl, 2008). The first set of atypical APDs were based off the benzodiazepine clozapine and this was followed by the development of risperidone, olanzapine and quetiapine, which more potently antagonize the serotonin 5-hydroxytryptamine (5-HT)<sub>2A</sub> receptor than the dopamine D2 receptor (Meltzer, 2004). Despite their success, atypical APDs can induce

severe metabolic disorders (i.e. metabolic syndrome, agranulocytosis, seizures, obesity, cardiovascular disease and Type II diabetes) (Reynolds & Kirk, 2010; Sharma et al., 2003). The atypical APDs developed were the benzamides, which selectively antagonize the D2/D3 receptor, have very low affinity for various other receptor types (Meltzer, 2004). Their non-specific binding characteristics have been shown to improve the negative symptoms and cognitive dysfunction associated with schizophrenia, while causing less of the metabolic side effects observed in previous atypical APDs (Jafari, Fernandez-Enright, & Huang, 2012; Mailman and Murthy, 2010).

#### **1.4.3** The Third Generation

The third generation APDs (ex. Aripiprazole) can be described as partial dopamine D2 receptor agonists that were developed to partially stimulate the receptor to a lesser extent than endogenous dopamine. This is contrary to the high D2 receptor affinity of typical antipsychotics in that it theoretically allows for safer competition with dopamine in hyperdominergic regions of the brain such as the striatum and dopamine receptor stimulation in hypodopaminergic regions such as the PFC (Perreault *et al.*, 2011). In reality, prior studies have shown variability in aripiprazole's agonist function across different cell lines, where in some cell lines it may act as a full agonist for D2-mediated inhibition of dopamine synthesis (Shapiro *et al.*, 2003). This observation led Mailman & Murthy, 2010 to propose the notion of "functional selectivity" in that a particular drug can have significantly different signalling through the same receptor and aripiprazole has been noted as an example of this. This type of pharmacodynamic approach may be important in future APD development as a drug capable of differential

signaling may be capable of preventing the activation of region specific pathways involved in the production of side effects, while still activating the same receptor pathways in a different region of the body to produce the desired clinical effect (i.e. the reduction of schizophrenic symptoms).

## 1.5 Allosteric Modulation using PLG and PAOPA

#### **1.5.1** Allosteric Modulation as the solution

Like that of the third generation of APDs, allosteric modulators for schizophrenia specifically activate particular pathways in a functionally selective manner (Conn, Christopoulos, & Lindsley, 2009). Instead of the ligand (or drug) binding to the orthosteric (active) site of receptor, allosteric modulators bind at an allosteric (regulatory) site on the receptor, which induces conformational changes at the orthosteric site that may affect ligand-receptor coupling or the modulation of orthosteric ligand binding affinity (Dyck *et al.*, 2011; Wang et al., 2009).



*Figure 2*. The difference in binding to the D2 receptor between traditional antipsychotic drugs and allosteric modulators.

On a positive note, allosteric modulators do not compete with endogenous ligands for orthosteric site occupation as they specifically target the allosteric site, only changing how the endogenous ligand reacts with its target receptor (Conn, Christopoulos, & Lindsley, 2009). These modulators have a ceiling as well due to the fact that their function is completely dependent upon the level of deviation from the physiological norm and are therefore more likely to be safer than orthosteric compounds that may overstimulate their target (Conn, Christopoulos, & Lindsley, 2009). A positive allosteric modulator will lead to an increase in the binding affinity, whereas a negative allosteric
modulator will lead to a decrease in the binding affinity of the endogenous ligand for its target receptor.

There have been several allosteric modulators developed for the treatment of schizophrenia including allosteric modulators for targeting the metabotrobic glutamate receptor subtypes mGluR2 (ly487379 and biphenyl-indanone A (BINA)) and mGluR5 (3,3'-Difluorobenzaldazine (DFB), N-{4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) and 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB)), nicotinic acetylcholine receptors as well as the muscarinic M4 receptor (Brady *et al.*, 2008; Buchanan *et al.*, 2008; Conn, Christopoulos, & Lindsley, 2009). The only real downside to this approach is that the desired clinical effects of these drugs rely upon the indirect normalization of dopaminergic function rather than direct dopamine receptor stimulation (Gill *et al.*, 2011; Schilstrom *et al.*, 2007; Wang *et al.*, 2007).

To the address the issues associated with long-term APD use, modulation of the allosteric (regulatory) site of the D2R has the potential to be more effective in the treatment of schizophrenia through the regulation of dopamine binding instead of prolonged activation (which would lead to the aforementioned side effects) (Dyck *et al.*, 2011; Mann *et al.*, 2010; Sharma *et al.*, 2003).

#### **1.5.2 PAOPA and Its Functionality**

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 treatment leads to the D2 receptor being placed in the high affinity state, resulting in the induction of GTPase activity and the inhibition of adenylyl cyclase (Mishra *et al.*, 1999). Through this positive allosteric modulation, endogenous L-prolyl-L-leucyl-glycinamide (PLG) and PAOPA been shown to promote D2 receptor internalization, therefore compensating for D2 hypoactivity in the prefrontal cortex and hyperactivity in the striatum (Mann *et al.*, 2010; Namkung *et al.*, 2009).

Based upon previous studies where PLG or its analogue PAOPA were given to PCP-induced schizophrenic rats, the negative symptoms of schizophrenia (asociality, rigidity, etc.) and some of the cognitive dysfunction has been shown to be attenuated (Beyaert *et al.*, 2013; Dyck *et al.*, 2011). Treatment with PAOPA has been shown to prevent both amphetamine-induced deficits in pre-pulse inhibition (model of negative symptoms and hyperlocomotion (model of positive symptoms) as well (Beyaert *et al.*, 2013).

PAOPA has been shown to be significantly safer than orthosteric site blockers in that it does not induce the metabolic or movement disorders seen in the long-term use of APDs (Sharma *et al.*, 2003; Tan *et al.*, 2013). Previous studies have also shown that the likelihood of the development of tardive dyskinesia in a rodent model (measuring vacuous chewing movements) is significantly reduced when schizophrenic rats were treated with PAOPA (Kapur & Seeman, 2000; Sharma et al., 2003). Interestingly, PAOPA has also demonstrated efficacy in preventing 1-methyl-4-phenyl-1,2,3,6tetrahydropyridine (MPTP)-induced movement disorders and the modulation of rotational

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behaviour in a rodent 6-hydroxydopamine (6-OHDA) lesion model of Parkinson's disease (Marcotte *et al.*, 1998; Mishra *et al.*, 1997; Ott *et al.*, 2000). All of the aforementioned traits make PAOPA an ideal drug for the treatment of schizophrenia.



Figure 3. The chemical structure of PAOPA.

## 1.6 Polar Drugs, the Blood Brain Barrier and Drug Carriers

## **1.6.1 Blood Brain Barrier Selectivity**

Polar molecules do not generally have high bioavailabilities within the brain after intranasal administration and due to the polar properties of the amide groups within the PAOPA molecule, PAOPA is not lipophilic (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 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 carrier's lipophilic character and molecular size (Dhuria, Hanson, & Frey, 2010). The drug also needs to be much smaller than 150 nm in size to pass the blood brain barrier (Gaumet *et al.*, 2008). This size is necessary 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).

#### 1.6.2 The Nanoparticle Advantage

A nanoparticle or microgel formulation would offer an effective and efficient means of overcoming the above challenges, resulting in a higher drug bioavailability within the brain (Illum, 2002). Nanoparticles increase 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 engineer slow degrading, uncharged, functionalized surface coatings that control drug release (thereby delaying drug metabolism), discourage particle aggregation (avoiding biological clearance mechanisms) and promote disease or cell-specific localization can help address the challenges of BBB transport (Dhuria, Hanson, & Frey, 2010; Sahoo & Labhasetwar, 2003; Singh & Lillard, 2009). Drug carrier development specialized for various routes of administration and drug polarity is integral for encapsulating drugs used in the treatment of a variety of CNS disorders (e.g. Schizophrenia, Parkinson's disease, etc.) (Sahoo & Labhasetwar, 2003).

## 1.7 Antipsychotic drug-loaded Drug Carriers

## **1.7.1** Drug Administration and the Intranasal Route

Nanoparticles have been tested in rodent models using intranasal, depot/injectable (subcutaneous, intramuscular, intraperitoneal and intravenous), and oral routes of administration to treat schizophrenia (Ereshefsky & Mascarenas, 2003; Kumar *et al.*, 2008). The availability of multiple routes of administration with nanoparticle drug carriers could provide patients and medical practitioners with the flexibility to choose a preferred route of administration for the patient (Ereshefsky & Mascarenas, 2003; Luppi *et al.*, 2010).

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 chosen when expedited action is desired 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).

In comparison, the intranasal route of administration has been found not only to be the fastest route to the brain, but to result in a higher drug bioavailability due to a greater CNS uptake than that of injection or oral administration of nanoparticle formulations in rats (Kumar *et al.*, 2008; Luppi *et al.*, 2010; Ugwoke *et al.*, 1999). There are many advantages to intranasal administration including the absence of drug degradation in the gastrointestinal tract and hepatic first pass metabolism, the drug carrier can be rapidly absorbed and a quick onset of action can be achieved (Chhajed, Sangale, & Barhate, 2011).

The uptake of these drug-loaded particles occurs via four potential mechanisms. The first of these mechanisms is referred to as the paracellular route, in which water soluble drugs <1000 Da in size are slowly and passively absorbed at the olfactory epithelium. (Chhajed, Sangale, & Barhate, 2011) The second is that of the lipoidal route or transcellar process in which lipophilic drugs or drug-loaded carriers tend to penetrate the lipid membrane at the olfactory epithelium. The final mechanism involves the active drug carrier mediated transport of drugs across the olfactory epithelial cell membrane through the opening of tight junctions or endocytosis through drug carrier-cell surface interaction (ex. Lectin-glucosamine interaction) (Chhajed, Sangale, & Barhate, 2011; Chen *et al.*, 2011). This surface interaction could potentially occur at two locations, the olfactory nasal epithelium (indirect route) or the olfactory nerve cells (direct route) as both are in close vicinity, yet both mechanistic pathways are incredibility complicated.

#### **1.7.2** The Indirect and Direct Intranasal Routes of Particle Uptake

The indirect route starts with the endocytosis of nanoparticles via the olfactory epithelial cells following binding to N-acetylglucosamine residues on the cell surface, which has been proposed (in other cell types) to travel through the cell as endosome-like structures (i.e. via transcytosis) and exit through the basal side of the cell via vesicle fusion/exocytosis potentially out into the subepithelial space or fluid (Fabis *et al.*, 2008; Mathison, Nagilla & Kompella, 1998; Patel *et al.*, 2012). The nanoparticle would then bypass the subepithelial connective tissues within this space travelling through the lamina propria to passively diffuse through the endothelial tight junctions of the nasal vascular bed (i.e. supplied via the external carotid and opthalamic artery), which are designed for

the rapid exchange of fluid or dissolved substances to later cross the blood brain barrier (Chhajed, Sangale, & Barhate, 2011; Mathison, Nagilla & Kompella, 1998; Patel et al., 2012). From here these particles would either travel across to the opposite side of capillary or potentially flow much futher to the Circle of Willis and travel more globally around the brain explaining the spread of dye-nanoparticles as well as the greater olfactory bulb concentrations of dye following intranasal administration, where the later would seem to occur due to greater regulation of neurovascular permeability via inflammatory cell types, serotonin, bradykinin, histamines and P2U (P2Y2) G-proteincoupled receptor pathways (Abbott, 2000). When travelling through the neural side, the particles would travel through the endothelial tight junctions to the basement membrane potentially followed by passage through the glial limitans gap junctions (Fabis et al., 2008; Pardridge, 2005). Following passage through the gap junctions into the cerebral spinal fluid (CSF), it is possible for the particles to bind to N-acetylglucosamine residues on the neuronal cell surface (potentially involved in neuronal cell interaction and plasticity) to be uptaken for use, degradation or to continue travel around the multitude of interconnected neuronal pathways within the brain (Kleene & Schachner, 2004). During this entire process it is also possible for the drug loaded within these nanoparticles to leak out, where it may continue to travel to the brain (provided it is non-immunogenic and/or lipophilic) or if already in the brain near its therapeutic site of action, exert its therapeutic effect (Chen et al., 2013).

The direct route potentially involves the receptor mediated endocytosis of nanoparticles via the olfactory receptor cells that extend into the nasal mucosa (Mathison,

Nagilla & Kompella, 1998; Pardridge, 2005; Patel et al., 2005). These uptaken nanoparticles could then travel directly up one of the cells (via transcytosis) of the olfactory receptor cell fascicles (receptor cell bundles) extending through the basal membrane, lamina propria, and pores in the cribiform plate to the olfactory bulb (Mathison, Nagilla & Kompella, 1998). The axons of these fascicles branch out to form glomerular tufts (or glomeruli), where the nanoparticles could exit the cell via exocytosis and cross the synaptic cleft to uptaken at the dendrites of the mitrial cells (Mathison, Nagilla & Kompella, 1998). The nanoparticles would then travel down the bundles of the mitrial cells present in the olfactory bulb that form the olfactory tract, which could take these nanoparticles along the ofactory tract projections to the prefrontal cortex, amygdala, thalamus and entohinal cortex (then projecting to the hippocampus) (Krettek & Price, 1977; Mathison, Nagilla & Kompella, 1998). From many of these regions there are a number of ways that the nanoparticles could reach the striatum (the site of action for many APDs). An example of this would be through the transport of the nanoparticles via glutaminergic neurons projecting from the prefrontal cortex to that synapse on D2 receptor expressing neurons within the striatum (Geffen, 2000). This pathway would therefore allow the nanoparticles to skip the exclusive blood brain barrier and make it to tissue containing the drugs target of interest in producing a clinical effect (see figure 4 for nasal anatomy).

It is imperative that the drug carrier chosen with the capability of traversing either of these pathways to ensure the success of transport of the drug carriers is maximized. There drug carriers must also be catered to the hydrophilicity or hyprophobicity of the

polar drugs being administered to treat schizophrenia in order to ensure maximal encapsulation efficiency and cost-efficacy.



*Figure 4:* Animation outlining the basic physiological structure of the human olfactory epithelium and its connection to the olfactory bulb.

## **1.7.3 PEG-PLGA Nanoparticles (Hydrophobic Polar Drugs)**

Nanoparticles are solid, vesicular particles in which a drug is dissolved, entrapped, adsorbed, attached and/or encapsulated (Sahoo & Labhasetwar, 2003). These drug carriers can be produced in varying sizes (10 to 1000 nm) based upon the preferred route of administration (Sahoo & Labhasetwar, 2003; Singh & Lillard, 2009). Copolymer polyethers used in nanoparticulate formulations such as methoxy PEG (mePEG) are very

lipophilic, non-immunogenic, can self assemble into miscelles in aqueous solutions with a 10 - 100 nm size range (depending on the length of PEG block polymers), and have been shown to have an excellent bioavailability within the brain (Avgoustakis *et al.*, 2003; Chen et al., 2011; Deng et al., 1995; Xia et al., 2011). Using mePEG in a copolymerization reaction with a D, L-lactide has also been shown to improve the brittleness and hydrophobic property of the poly D,L-lactic-co-glycolic acid (PLGA) component of the molecule, which aids in the formation of thermodynamically stable copolymers (Deng et al., 1995; Zhang et al., 2004). PLGA is being used due to its nontoxic, biodegradable properties and for its ability to interact with the dichloromethane organic phase (see figure 1). The PEG-PLGA copolymer coating also causes the nanoparticles to display a longer half-life in the blood due to little non-specific interaction with biological components (Chen et al., 2011; Deng et al., 1995; Rosler, Vandermeulen & Klok, 2001). To date there are only two studies that specifically involve the intranasal administration of APDs. Olanzapine-loaded PLGA nanoparticles of size 100-200 nm were demonstrated to achieve low to moderate drug encapsulation and high neural bioavailability in vivo compared to other routs of administration (Seju, Kumar, & Sawant, 2011). Since these particles lack a coating such as PEG to stabilize the particles through steric interactions, the PLGA particles would likely be subject to aggregation and removal via nasal clearance mechanisms. In another study, risperidone-loaded solid lipid nanoparticles (SLNs) with particle sizes near 150 nm limit for passive transport across the BBB were reported to facilitate moderate drug encapsulation (Patel *et al.*, 2011). Improved APD loaded nanoparticle formulations need to exhibit smaller particle sizes

and lower surface charges (to allow greater transport across the BBB) while facilitating high drug encapsulation efficiencies to account for potential premature drug loss due to nasal clearance mechanisms (Illum, 2003). Based on these requirements, poly(ethylene glycol)–block-poly (D,L-lactic-co-glycolic acid) (PEG-PLGA) copolymer nanoparticles that feature a PEG shell around the PLGA nanoparticle core are ideal for intranasal administration of hydrophobic polar drugs (see figure 5). The PEG shell has been demonstrated to prevent the nanoparticle aggregation typically seen with uncoated PLGA nanoparticles upon contact with the nasal mucosa (Ereshefsky & Mascarenas, 2003). In addition, these particles can be prepared quite reliably at a size <150 nm, prolonging circulation time and inhibiting nanoparticle uptake by the mononuclear phagocytic system (Jaeghere *et al.*, 1999).



*Figure 5:* Animation outlining the basic structure of the *Solanum Tuberosum* lectin functionalized Haloperidol-loaded PEG-PLGA nanoparticles

## **1.7.4 POEGMA Microgels (Hydrophilic Polar Drugs)**

Microgels possess many of the same advantages needed to overcome BBB issues as they are easy to synthesize, monodisperse in size, offer long-term stability, shelf-life, and biocompatibility (Smeets & Hoare, 2013). 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 highly hydrophilic water-swollen networks that present a low interfacial energy under biological conditions, reducing opsonisation (non-specific interactions with proteins that promote ingestion by phagocytes), increasing their bioavailability and reducing the likelihood of an immune response (Smeets & Hoare, 2013). 3-(4,5 dimethylthiozol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays have shown that microgels are non-toxic, in particular if higher amounts of PEG were incorporated in the microgel design (Peng et al., 2012). 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·mL-1 while paxlitaxel-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, Maitland, & Hoare, 2011). In addition, these formulations have demonstrated excellent encapsulation efficiencies and loading capacities (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. This demonstrates that microgels are an ideal candidate for the encapsulation and release of hydrophilic CNS drugs (particularly APDs) to their targets within the brain, which is generally at pH 7.4 as well as 37°C. No microgel formulation has yet been used for the encapsulation of CNS therapeutics let alone APDs or even been studied to determine neural bioavailability though here we propose a cartoon of its structure (see Figure 6) (Smeets & Hoare, 2013). There is a great degree of potential for microgel formulations to be produced at sizes <150 nm, which is necessary for passage across the blood brain barrier (Gaumet et al., 2008). This size is necessary 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). This could increase the likelihood of hydrophilic APDs reaching their desired targets within the brain.



*Figure 6:* Animation outlining the basic structure of the *Solanum Tuberosum* lectin functionalized PAOPA-loaded POEGMA microgels

## **1.8 Cell Targeting Specific to Intranasal Administration**

Another way to increase the likelihood of reaching the brain through the intranasal route of administration is through the use of *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 surface maleimide residues on the PEG-PLGA particle surface or the acrylic acid residues on the POEGMA microgel surface with *Solanum tuberosum* lectin (STL), the APD drug carriers should be more effective at selectively binding to the N-acetylglucosamine residues highly expressed on the nasal epithelial and neuronal cell membranes (Chen *et al.*, 2011; Lundh, Brockstedt, & Kristensson, 1989; Pastor *et al.*, 1992; 2i). This selective binding

has been previously shown to lead to increased nasal epithelial cell uptake and improved nanoparticle bioavailability within the brain (Chen et al., 2011; Vila et al., 2005). Prior research has also shown that STL activity is unchanged upon heating below 50°C and remains relatively stable over a large pH range (i.e. pH 4-10) (Chen et al., 2011). Such targeting has not yet been demonstrated using APD-loaded PEG-PLGA nanoparticles for the purposes of more effective at N-acetylglucosamine binding specific to the intranasal route of administration (Chen et al., 2011; Lundh, Brockstedt, & Kristensson, 1989; Pastor et al., 1992). In a similar fashion, antibodies have also been immobilized onto microgels, but 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). This would be the first time that microgels have been functionalized for cell-targeting for the nasal route of administration for APDs as well. The above properties of the lectin target, coupled with the relatively small nanoparticle surface charge, should inhibit aggregation, reduce interaction with the mucin molecules and thus avoid the nasal clearance mechanisms (Illum, 2003).

# **2 OBJECTIVES, HYPOTHESES AND SIGNIFICANCE**

The *objective* of this project was to develop a novel microgel drug carrier for the hydrophilic drug PAOPA and a nanoparticle drug carrier for the hydrophobic drug Haloperidol that can be effectively administered via an intranasal route using a newly designed intranasal spray device. We hypothesize that this formulation will increase treatment efficacy and allow for a reduction in the dosage necessary to produce a clinical effect.

2.1. To develop functionalized Poly [oligo(ethylene glycol) methyl ether methacrylate] (POEGMA) microgels carrying PAOPA engineered to effectively bind to N-acetylglucosamine residues on the nasal epithelial cell membrane

PAOPA has been shown to increase dopamine binding to the D2 receptor by increasing the high affinity state of the D2 receptors (D2<sup>High</sup>) relative to the low-affinity state (D2<sup>Low</sup>), while having no effect on antagonist binding (Dyck *et al.*, 2011; Mann *et al.*, 2010). Increased dopamine binding causes a compensatory response in which the D2 receptor translocates from the dopaminergic cell membrane into the cytosol via endocytosis in order to prevent receptor overstimulation (Namkung *et al.*, 2008). Since polar molecules such as PAOPA do not generally have high neural bioavailabilities following intranasal administration due to mucin-drug interactions at the olfactory epithelium, PAOPA would be more susceptible to clearance via the beating nasal cilia (Meador-Woodruff *et al.*, 1996). A microgel nanoparticle formulation would offer an effective means of overcoming this challenge, resulting in a higher bioavailability within the brain (Meador-Woodruff *et al.*, 1996). The functionalization of the particle surface

with STL may potentially allow the PAOPA-loaded microgels to be more effective at selectively binding to the N-acetylglucosamine residues highly expressed on the nasal epithelial cell membrane and being uptaken via the nasal epithelial cells (Chen *et al.*, 2011). This approach has been demonstrated to increase uptake via nasal epithelial cells and improve nanoparticle bioavailability within the brain (Kim, 2008). Surface charge can also have a dramatic effect on nanoparticle uptake, whereby cationic charges have been shown to generate a greater immune response and increase the rates of phagocytic uptake when compared to neutral or anionic nanoparticles. Nanoparticle surface charges between -10 and +10 mV having been suggested as optimal for preventing non-specific protein adsorption (Swami *et al.*, 2012).

We hypothesized that: (1) the PAOPA-loaded microgels may be small enough to cross the blood brain barrier with a non-immunogenic surface charge; (2) the PAOPA-loaded microgels may be successfully functionalized with STL; (3) the PAOPA-loaded microgels will potentially be non-toxic in both cells lines.

# 2.2. To develop a multi-dose intranasal spray device administering aerosolized metered doses of liquid nanoparticulate drug formulation in a rodent model

Drugs delivered travel via an intranasal route to the brain and diffuse through the blood brain barrier at a faster rate than that of injectable or orally administered drugs (Illum, 2002; Ugwoke *et al.*, 1999). Currently, most studies of intranasal drug administration using nanoparticles involve the direct pipetting of formulation into the nasal cavity of the anaesthetized rodent (Chen *et al.*, 2011; Gao *et al.*, 2011; Xia *et al.*, 2011). This is an inefficient model for studying intranasal administration as it will lead to a higher incidence of nasal drip down the nasal cavity into the pharynx (i.e. throat), where it will eventually enter the stomach and be degraded by the stomach acid (Illum, 2003). In order to combat this, a single-dose intranasal spray will be administered to rats by the experimenter in a controlled metered dose through loading the reservoir with the exact dose needed for the rat being tested. I have worked with Zhu from the lab of Dr. Ravi Selvaganapathy in the mechanical engineering department to produce an electronically controlled nasal spray device. This device will use manually loaded dosing (i.e. a central reservoir that can be filled with the subject specific dose using a pipette) and will use an external pressure source separate from that of the drug reservoir allowing control over the amount of pressure used to deliver the drug-loaded nanoparticles. The device will be connected to a circuit board for solenoid valve control through the program LabVIEW for automation (Gabrio, Stein, & Velasquez, 1999).

We hypothesized that: (1) the haloperidol tissue concentrations (i.e. in the olfactory bulb and striatum) may be highest in the animals using the device over that of the pipette for intranasal administration; (2) the haloperidol tissue concentrations may be highest in the striatum due to the high density of the D2 receptor (Weiner & Brann, 1989).

2. 3. To validate that the POEGMA microgels cross the blood brain barrier following administration via the intraperitoneal route.

There has never been a microgel or microgel formulation used for the encapsulation of CNS therapeutics let alone APDs. To go a step further, these formulations have not yet been shown to cross the BBB in any type of study on their

neural bioavailability following any route of administration (de Jong & Born, 2008). There is a great degree of potential for microgel formulations to be produced at sizes <150 nm, which is necessary for passage across the BBB (Gaumet *et al.*, 2008). This size is necessary for passive diffusion of solid nanoparticles 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). This may potentially vary with microgels of various sizes as these drug carriers are more flexible than that of solid drug carriers such as the PEG-PLGA nanoparticles, in which particles >150 nm may be able to squeeze through endothelial tight junctions to pass through the BBB (Gaumet *et al.*, 2008). This could dramatically increase the likelihood of hydrophilic APDs in reaching their desired targets within the brain. In order to do this, the use of a fluorescent tag (i.e. rhodamine) to the POEGMA polymer itself would allow the tracking of these microgels throughout the body and within the blood *in vivo*.

We hypothesized that: (1) the smaller rhodamine microgels (i.e. 100 nm microgels) will potentially pass through the blood brain barrier and be detectable within the brain; (2) the smaller rhodamine microgels (i.e. 100 nm microgels) could have the greatest tissue concentrations within the major organs (i.e. liver, lungs, spleen and kidneys); (3) the larger microgels may have the greater blood serum microgel concentrations due to an inability to be uptaken by tissues or pass the BBB.

#### 2.4. Significance

This study had several unique features including: (1) The first use of a therapeutic approach that possesses *Solanum tuberosum* lectin functionalized POEGMA microgels,

which are much more likely to cross the BBB through their increased interactions with nasal epithelial cells and their ability to diffuse into the blood based upon their size. (2) This treatment approach brings in a new strategy for patient administration of antipsychotic drugs using a metered single-dose nasal spray device, which gives the schizophrenic patient a choice over the common injection or oral treatment options. (3) These functionalized PAOPA-loaded microgels will not only have the potential to attenuate the positive and negative symptoms, but some of the cognitive dysfunction involved in schizophrenia at a lower dose than that of unencapulated PAOPA. (4) This study provides an original way modulate the dopamine D2 receptor via allosteric modulation compared to traditional antipsychotic drugs that target the orthosteric site of the dopamine D2 receptor, which should prevent the development of movement disorders associated with antipsychotic use such as tardive dyskinesia.

# **3 METHODOLOGY**

## **OBJECTIVE 1**

## **3.1. Hydrophilic POEGMA microgel synthesis**

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  $\mu$ L, 0.3 mmol), acylic acid (528  $\mu$ L, 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).

## **3.2. POEGMA microgel surface functionalization**

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 45,000 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 45,000 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.

#### 3.3. POEGMA and STL-POEGMA microgel drug loading

Either a 25 mg solution of POEGMA or STL-functionalized POEGMA microgels were transferred to 3 mL ultracentrifuge tubes and subjected to 45,000 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 45,000 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).

## 3.4. Microgel characterization, concentration and lectin conjugation density

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 (see Section 3.2.). The conjugation efficiency was calculated using the equations described by Chen *et al.*, 2011.

 $Conjugation efficiency = (Amount of STL added - Amount of unconjugated STL) \times 100\%$ Total amount of STL added

### 3.5. In vitro release of PAOPA from POEGMA and STL-POEGMA microgels

*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

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

PAOPA-loaded micgrogels 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-lyzers. 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).

# **3.6.** Determination of encapsulation efficiency, drug loading capacity, and *In vitro* PAOPA release

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  $\mu$ L 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;  $\lambda = 215$  nm for PAOPA). PAOPA was run using the Xbridge (C18) (5  $\mu$ m, 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 M.Sc. Thesis - J. Piazza; McMaster University – Neuroscience.

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:

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

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

## 3.7. PAOPA, POEGMA and STL-POEGMA microgel cytotoxicity studies

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 modified from 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<sup>4</sup> 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  $\mu$ L. 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  $37^{\circ}$ C and following the incubation, the MTT was removed from each well. To each well 100 µL dimethyl sulfoxide (DMSO) was then added to solubilise 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 (%) = 
$$\frac{Absorbance_{Polymer Solution}}{Absorbance_{Blank}}$$

#### **3.8. Statistical analyses**

All data analyses were performed using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA, USA). Before any analyses were performed, outlier detection was performed using the GraphPad Outlier Tool. All significance levels were defined as p < 0.05.

## 3.8.1 The effect of surface STL-functionalization on microgel size

A paired Student's t test was conducted to evaluate the relationship between microgel size and STL functionalization to determine whether functionalization significantly affects particle size.

## **3.8.2** The effect of surface STL-functionalization on microgel surface charge

A paired Student's t test was conducted to evaluate the relationship between zeta potential and STL functionalization to determine whether functionalization significantly affects particle size.

#### 3.8.3 The effect of surface STL-functionalization on microgel drug loading

A paired Student's t test was conducted to evaluate the relationship between encapsulation efficiency and STL functionalization to determine whether functionalization significantly affects particle size.

#### **OBJECTIVE 2**

# 3.9. Animals

All animals were housed and tested in compliance with the guidelines described in the Guide to Care and Use of Experimental Animals (Canadian Council on Animal Care, 1984, 1993). Twenty four male Sprague-Dawley rats were utilized for all experiments for the following reasons: 1) the use of male rats over female rats prevents potential complications as a result of the female estrous cycle and 2) this strain is most commonly used within the investigation of the effect of psychotropic drugs on animal behavior (Geyer et al. 2001). The rats were individually housed under constant temperature and humidity with a 12-h light/dark cycle and allowed ad libitum access to food and water. Colony temperature was maintained at  $21\pm1^{\circ}$ C and all experiments were conducted during the day cycle. All animal procedures were approved by the Animal Research Ethics Board, McMaster University, Hamilton, Ontario, Canada.

## 3.10. Preparation of PEG-PLGA nanoparticles

PEG-PLGA nanoparticles were prepared using a mixture of Mal-PEG-PLGA and Me-PEG-PLGA using the emulsion/solvent evaporation technique adopted from Chen et al., 2011. Briefly, a 9:1 mass ratio of Me-PEG-PLGA (22.5 mg) and Mal-PEG-PLGA (2.5 mg) was dissolved in 2.5 mL dichloromethane (DCM). The solution was emulsified by sonication on ice (200 W, two 30s intervals) in 20 mL of 1% polyvinyl alcohol (PVA) solution using a PCU-2-110 homogenizer (Kinematica Tech., Switzerland). The DCM was immediately evaporated at 40°C under reduced pressure using Buchi 011 RE 121 rotary evaporator (Buchi, Germany). The nanoparticles were subjected to centrifugation at 14,000 rpm for 45 min (4°C) using a J2-21M/E centrifuge (Beckman Coulter, USA) equipped with a J-20 rotor and then resuspended in 1 mL of deionized water. Haloperidol loaded nanoparticles were prepared using the same method except that 3.5 mg of drug was added to the DCM solution prior to emulsification. The supernatant following centrifugation of haloperidol-loaded nanoparticles was also removed for analysis of residual haloperidol concentration. Following rotary evaporation, the round bottom flask containing the haloperidol-loaded nanoparticles was washed with 2 mL of methanol containing 2% acetic acid and collected for later analysis. The obtained drug loaded nanoparticles were then purified using a Sepharose CL-4B column ( $1 \times 20$ cm) eluted with 0.05 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer pH 7.0 containing 0.15M NaCl to remove unentrapped drug and the supernatant from the fractions obtained from column purification were kept for later analysis.

### 3.11. Preparation of STL functionalized PEG-PLGA nanoparticles

STL was thiolated using 2-iminothiolane dissolved in 100  $\mu$ L of 0.15 M pH 8 borate buffer (supplemented with 0.1 mM Ethylenediaminetetraacetic acid (EDTA) at a molar ratio of 1:32 STL:maleimide for 60 min at room temperature. The thiolated STL reaction solution was subjected to a Hitrap<sup>TM</sup> desalting column (Amersham Pharmacia Biotech AB, Sweden) and eluted with 0.01M HEPES pH 7.0 containing 0.15M NaCl. The fractions were collected and the thiolated STL concentration was determined using UV/visible spectrophotometry at a wavelength of 412 nm with Ellman's reagent. The coupling procedure was performed by mixing the thiolated STL with the nanoparticles (from section 3.12.) using a 9:1 molar ratio (thiolated STL:mal-PEG-PLGA) in 1 mL deionized water at room temperature for 8 h. The nanoparticles were then collected via centrifugation at 14,000 rpm for 45 min at 4°C and washed three times with deionized water. The supernatant was removed for further analysis.

#### 3.12. Nanoparticle characterization, concentration and lectin conjugation density

The mean diameter of the nanoparticles was determined by DLS analysis using the Malvern Zetasizer Nano-ZS (Malvern, UK). The zeta potential of the nanoparticles was measured using the Zeta 90 Plus Zeta Potential Analyzer (Brookhaven Instrument Corp., USA). The morphological examination of the nanoparticles was performed using a JEM-1200ex TEM (Peabody, USA). Nanoparticle concentration was determined using NTA with a LM 20 NanoSight (Wiltshire, United Kingdom). The quantity of STL conjugated to the surface of the nanoparticles was determined using the bicinchoninic acid (BCA) method by measuring the concentration of unconjugated STL within the

supernatant following the centrifugation step (see Section 3.14.). The conjugation efficiency was calculated using the same above equations.

# **3.13.** Determination of nanoparticle encapsulation efficiency, drug loading capacity and *in vitro* release

In vitro release experiments of haloperidol from nanoparticles were performed at  $37^{\circ}$ C in both acetate buffer (154 mM, pH 4.5) and PBS + 0.1% tween 80 (10 mM, pH 7.4) to evaluate the APD leakage over a 96 h (5 day) incubation period. The pH 4.5 acetate buffer was selected to represent the pH of the endo-lysosomal compartment, with the additional advantage of facilitating higher drug solubility relative to the 10% solubility limit of haloperidol in PBS and the pH 7.4 phosphate buffer was selected to represent the pH of the cellular/extracellular compartment with the Tween surfactant component to simulate the cell membrane (Budhian, Siegel & Winey, 2005; Wibo & Poole, 1974). The entire batch of non-functionalized or STL-functionalized haloperidolloaded nanoparticles 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 acetate buffer (154 mM, pH 4.5) or PBS + 0.1% tween 80 (10 mM, pH 7.4). The samples were placed into a beaker in an incubator at 37°C at 100 rpm after closing the float-a-lyzers. 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) to determine haloperidol release and the removed volume was replaced with fresh 2 mL of acetate buffer (154 mM, pH 4.5) or PBS + 0.1% tween 80 (10 mM, pH 7.4).

The amount of haloperidol encapsulated was quantified via HPLC by difference, measuring the total loss of haloperidol at each step during nanoparticle synthesis as opposed to after synthesis. This approach avoids the requirement of complete dissolution of the drug loaded nanoparticles to measure loading as described in the prior literature, which due to solvent incompatibilities in dissolving the polymer and the drug can result in significant overestimates in drug loading (Budhian, Siegel, & Winey, 2010). The nanoparticle drug entrapment efficiency and drug loading capacity for haloperidol was determined using HPLC (Davda & Labhasetwar, 2002); Igarashi et al., 1995). A sample injection volume of 20 µL was used for haloperidol samples, measured using a Waters 2695 separation module (Model SM4; Waters Corporation, Canada) consisting of a Waters 2489 ultraviolet detector (Model 246;  $\lambda = 250$  nm for haloperidol). Haloperidol was run using the NovaPak C18 column (4  $\mu$ m, 150 mm  $\times$  3.9 mm), using a column temperature of 35°C and a flow rate of 1 mL/min. The separations for haloperidol were achieved using a mobile phase with a 57.2:22:20:0.8 (w/v/v/v) ratio of 0.01M ammonium acetate: methanol: acetonitrile: acetic acid (Igarashi et al., 1995). The DLC and EE were calculated using the same above equations.

# **3.14.** Development of a single-dose intranasal spray device administering aerosolized metered doses of liquid nanoparticulate drug formulation

Using device parameters designed using Autocad Inventor 2012 software (Autodesk, USA), a nasal spray device was constructed out of Visijet ex200 (i.e. acrylic) using a ProJet HD 3000 3D printer (3D Systems, USA). A thin grated detachable nozzle 10 mm long (inner diameter: 1mm; Outer Diameter: 1.5mm) was developed for optimal

drug delivery to the nasal cavity as the average anterior-posterior length of the rat nasal cavity is 7.0-9.1 mm (Gross et al., 1982). This was done so the nozzle would be small enough to fit within the rodent nasal cavity without causing tissue damage or the obstruction of breathing (Gross et al., 1982). This device uses a central reservoir adjacent to the spray nozzle that can be loaded with nanoparticulate or microgel solution for specific single-dose volumes (i.e. the reservoir is loaded with the dose desired for intranasal administration using a pipette in order to meter the dose). The nasal spray device uses flow-focusing in order to prevent surface tension from occurring within any of the channels within the device by splitting the flow of pressure from the pressure source into three channels (See Figure 14). Two of these channels split and then reconverge at the nozzle to prevent surface tension once the nanoparticle drug solution has reached the intermediate space between the nozzle and the reservoir. The third channel uses pressure to not only push down on the nanoparticle solution to move it towards the nozzle, but to also prevent back pressure and backflow of the solution back into the reservoir. Through the use of an external pressure source separate from that of the drug reservoir, the device can use high pressure to aerosolize the solution into a single spray to deliver the drug-loaded nanoparticles. The external pressure source is connected to the device at a 3 mm gas channel port that later splits into the first two channels mentioned. The flow of gas from the E size  $O_2$  tank through the device is controlled by an electronically controlled 3-way normally closed manifold solenoid valve (SM10MM-30-12-3) (Humphrey, USA) connected to a circuit board and programmed through the program LabVIEW for automation (Gabrio, Stein and Velasquez 1999).

# **3.15.** Spray distribution of the single-dose intranasal spray device administering aerosolized metered doses of liquid nanoparticulate drug formulation

In order to determine the surface area of liquid created by the spray, there was 100  $\mu$ L of either water, PEG-PLGA nanoparticles or PEG-PLGA nanoparticles plus 2% Methylene blue loaded into the reservoir of the device using a pipette. Using the device under approximately 1 barr of pressure, the drug solution was aerosolized for 30 s either 5 mm or 10 mm (i.e. 1 cm) in front of a vertical flat surface containing a sheet of paper. The largest possible measure of diameter from each spray surface area measurement on the sheet of paper was taken in order to account for potential error resulting from not using an automated integration program to measure spray surface area.

## **3.16.** Catalepsy testing

During each experimental session, the rats were transferred to a sound-attenuated room and allowed to habituate to the environment for at least one half hour prior to drug treatments. The front paws of the rat were gently placed in an extended position on a horizontally mounted metal bar that was 10 cm above a wooden platform, and the amount of time spent maintaining this abnormal position was measured. Rats were given empty nanoparticles pipetted intranasally in an anaesthetized state, haloperidol loaded STL-nanoparticles intranasally via the nasal spray device (6 rats/group). IN administration was performed on unanaesthetized or lightly anaesthetized rats in which one experimenter would hold the rat and the nanoparticle formulations were alternately dropped slowly into the nostrils of the rodent over a period of 2 min. The nasal

spray device was not used on unanaesthetized rats as it was quite stress inducing and caused shifting behavior that had the potential for breaking the acrylic nozzle leading to possible injury. All rats receiving haloperidol treatments received a dose of 2 mg/kg only once. All tests and injections were carried out between 9 am to 5 pm daily. To ensure enough drug had been released, the animals were tested in triplicate with 30 second intervals between tests an hour following drug administration, and the maximum intensity of the cataleptic response was recorded in seconds (Costain *et al.*, 1999). Rats were given a rated score (1-6) based upon time spent on the bar (1=0-10s, 2=11-20s, 3=21-30s, 4=31-40s, 5=41-50s or 6=51-60s) and the minimum cut off required for a response to be considered cataleptic was when both paws were on the bar for at least 20s or a score of at least 3 (Adams *et al.*, 1997).

#### **3.17.** Tissue analysis

Tissue samples from the striatal region of the basal ganglia, which is enriched in dopamine receptors, and the olfactory bulb were removed following 1 hour post-catalepsy testing for individual analyses of region-specific haloperidol concentration (Igarashi *et al.*, 1995; Weiner & Brann, 1989). Each of the tissue samples was prepared as described by Igarashi *et al.*, 1995. Samples were homogenized using a sonic 300 dismembrator (Artek Inc., USA) in 3 volumes of ice-cold 1.15 % potassium chloride (KCI) solution on ice and were then mixed with 2 volumes of 2 % (v/v) acetic acid in methanol. Samples were subjected to centrifugation at 9500 RPM (4°C) using a 5415 R, 230 V/50-60 Hz centrifuge (Eppendorf, Germany) for 10 minutes to remove all protein and tissue from the

extracted haloperidol. Following haloperidol extraction, 20  $\mu$ L aliquots of the supernatant were analyzed by HPLC as described above.

#### **3.18. Statistical analyses**

# **3.18.1** The effect of distance on spray surface area using a single-dose metered nasal spray device

A one way repeated measures 2 x 3 Analysis of Variance (ANOVA) with a Bonferroni post-hoc test was conducted to evaluate the relationship between distance and drug solution type with respect to spray surface area following aerosolization. The first factor for the ANOVA was distance with two levels (5 mm away from the vertical surface or 1 cm away from the vertical surface). The second factor for the ANOVA was aerosolized solution type with three levels (water, PEG-PLGA nanoparticles or PEG-PLGA nanoparticles with methylene blue). The dependent variable for both factors was spray surface area on the vertical surface.

#### 3.18.2 The effect intranasal administration type on catalepsy rating

A one way ANOVAwith Turkey's post-hoc test was conducted to evaluate the relationship between drug treatment and the degree of cataleptic response in rats. The between-subjects factor for the ANOVA was drug treatment with four levels (empty nanoparticles pipetted intranasally with anaeasthesia, haloperidol-loaded STL-nanoparticles pipetted intranasally without anaesthesia, haloperidol-loaded STL-nanoparticles pipetted intranasally with anaesthesia and haloperidol-loaded STL-nanoparticles administered intranasally via the nasal spray device) and the dependent variable was catalepsy rating.

## 3.18.3 The effect of intranasal administration type on tissue uptake

A one way nonparametric ANOVA with Turkey's post-hoc test was also conducted to evaluate the relationship between drug treatment and haloperidol tissue concentration separately for two distinct brain regions (olfactory bulb and striatum) in rats. The between-subjects factor for the ANOVA was drug treatment with four levels (empty nanoparticles pipetted intranasally with anaeasthesia, haloperidol-loaded STLnanoparticles pipetted intranasally without anaesthesia, haloperidol-loaded STLnanoparticles pipetted intranasally with anaesthesia and haloperidol-loaded STLnanoparticles administered intranasally via the nasal spray device) and the dependent variable was the tissue haloperidol concentration (µg haloperidol/g brain tissue).

### **OBJECTIVE 3**

### 3.19. Animals

Twenty male Sprague-Dawley rats were obtained from Charles River weighing between 275 - 300 g (Charles River Laboratories, Wilmington, MA). The rats were individually housed under constant temperature and humidity with a 12-h light/dark cycle and allowed ad libitum access to food and water. Colony temperature was maintained at  $21\pm1^{\circ}$ C and all experiments were conducted during the day cycle. All animal procedures were approved by the Animal Research Ethics Board, McMaster University, Hamilton, Ontario, Canada.

### 3.20. Rhodamine-labelled POEGMA microgel synthesis
Rhodamine-labelled POEGMA microgels were synthesized via precipitation polymerization in water at 90°C. Diethylene glycol methacrylate (0.5 g), Ethylene glycol dimethylacrylate (EGDMA) (30  $\mu$ L), acylic acid (57  $\mu$ L) and three different amounts of sodium dodecyl sulfate (40 mg = 100 nm, 20 mg = 150 nm, 6 mg = 250 nm) were added (depending on desired particle size) to a two-neck round bottom flask and distilled deionized water (150 mL) was added. Solutions of deionized water were saturated with 4.5 mg of rhodamine 6G based upon its solubility in water (0.32 mg/mL). 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 (20 mg 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 at pH = 7.4. The acrylic acid content was determined by titration with sodium hydroxide using a PC-Titrate system (Mandel Scientific Co., Canada).

### **3.21. Rhodamine-labelled POEGMA microgel treatment, perfusion and tissue extraction**

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.

### **3.22. Rhodamine-labelled POEGMA and STL-POEGMA microgel tissue uptake studies**

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 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 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).

### **3.23. Rhodamine-labelled POEGMA and STL-POEGMA microgel blood serum studies**

Blood samples were transferred to eppendorf tubes and subjected to 3000 RPM for 5 min using a 5415 R, 230 V/50-60 Hz centrifuge (Eppendorf, Germany). The serum supernatant was transferred to new tubes and stored at  $-20^{\circ}$ 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).

#### **3.24. Statistical analyses**

A two way ANOVA with Turkey's post-hoc test was conducted to evaluate the relationship between rhodamine-labelled microgel size and brain tissue type concentration. The first factor (microgel size) is between-subjects and contains four levels (non-labelled 100 nm, 100 nm, 150 nm and 250 nm). The second factor (brain tissue type) contained three levels (PFC, striatum and cerebellum). The dependant variable is the tissue concentration following treatment with differing sizes of rhodamine-labelled microgels.

A two way ANOVA with Turkey's post-hoc test was conducted to evaluate the relationship between rhodamine-labelled microgel size and major organ tissue concentrations. The first factor (microgel size) is between-subjects and contains four

levels (non-labelled 100 nm, 100 nm, 150 nm and 250 nm). The second factor (major organ tissue) contained four levels (liver, lungs, spleen and kidneys). The dependant variable is the tissue concentration following treatment with differing sizes of rhodamine-labelled microgels.

A one way nonparametric ANOVA with Turkey's post-hoc test was also conducted to evaluate the relationship between rhodamine-labelled microgel size and total microgel blood serum concentrations. The between-subjects factor for the ANOVA was microgel size with three levels (100 nm, 150 nm and 250 nm) and the dependent variable was the total blood serum microgel concentration (µg microgel/total serum volume).

#### **4 RESULTS**

## 4.1. POEGMA microgel characterization, concentration and lectin conjugation density

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 deswelling due to the low pH (pH 4.5) of the uranyl acetate stain, which may have also led to some aggregation resulting from microgel uranyl acetate interactions (Figure 7). Nanoparticle tracking analysis indicated a nanoparticle concentration of  $3.3-3.6 \times 10^{12}$ 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  $85.5 \pm 1.6$  % was achieved, resulting in a surface density of STL on the nanoparticles of  $429.8 \pm 7.9$  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.



Figure 7: TEM images of the nanoparticles at 150,000x magnification (POEGMA

microgels + PAOPA on the left and STL-POEGMA microgels + PAOPA on the right)

*Table 1*. Microgel size, polydispersity index and zeta potential for each of the microgel samples prepared in this study.

Microgel Type and Drug	<b>Microgel Size</b>	Polydispersity	Zeta Potential
Encapsulated	( <b>nm</b> )	Index (PDI)	( <b>mV</b> )
Empty Microgels	130.2 <u>+</u> 1.75	0.243 <u>+</u> 0.003	-13.6 <u>+</u> 1.0
Empty STL-Functionalized	121.6 <u>+</u> 1.53	0.132 <u>+</u> 0.005	-16.8 <u>+</u> 0.7
Microgels			
PAOPA-Loaded Microgels	141.7 <u>+</u> 1.15	0.132 <u>+</u> 0.008	-14.4 <u>+</u> 1.9
STL-Functionalized	114 <u>+</u> 0.33	0.156 <u>+</u> 0.061	-16.9 <u>+</u> 0.9
PAOPA-Loaded Microgels			

## 4.2. Drug quantification and release from functionalized and non-functionalized POEGMA microgels

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 moderate 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 indicates the loss of some the POEGMA microgels during the additional 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).

*Table 2*. Comparison of microgel concentration, encapsulation efficiency, drug loading capacity and cumulative release *in vitro* for each of the microgel samples prepared.

Microrogel Type and Drug Encapsulated	Microgel Concentration (particles/mL)	Encapsulation Efficiency (%)	Drug Loading Capacity (%)
Empty Microgels	$3.47 \ge 10^{12}$	-	-
Empty STL-Microgels	$3.30 \ge 10^{12}$	-	-
PAOPA-Loaded Microgels	$3.57 \ge 10^{12}$	$33.6\pm2.4\%$	$0.39\pm0.03\%$
STL-Functionalized	$3.60 \ge 10^{12}$	$30.2\pm1.9\%$	$0.35\pm0.03\%$
PAOPA-Loaded Microgels			

4.3. In vitro drug release study from STL-functionalized and non-functionalized

**PAOPA-loaded POEGMA microgels** 

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 8, the total observed *in vitro* release for haloperidol in pH 7 phosphate buffer at 37 °C was 100% of loaded drug from both the STL-microgels and the non-functionalized microgels after a 96 hr incubation period. There also wasn't much difference observed in drug leaching between non-functionalized over STL-functionalized PAOPA-loaded microgels (Figure 8). This result suggests that minimal drug is lost from the microgels during the period over which they would be transported to the target tissue.



*Figure 8. In vitro* leakage of PAOPA from microgels and STL-functionalized microgels in 10 mM PBS pH 7.4 (n = 3).

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

In the RPMI 2650 Nasal septum carcimoma cells, there was little decrease in cellular viability after 24 h of treatment with the PAOPA, POEGMA or STL-POEGMA microgel treatments compared to the untreated control cells (Figure 9). Though there was a concentration dependent decrease in cell viability with increasing treatment concentrations, this was only a 10-15% decrease at exceptionally high treatment concentrations (i.e. 2 mg/mL).



*Figure 9.* RPMI 2650 Nasal Septum Carcinoma cell viability following 24 hr exposure to either PAOPA, POEGMA or STL-POEGMA microgels and incubation at  $37^{\circ}$ C (*n* = 4/treatment)

In the SHS 5Y Neuronal cells, there was little decrease in cellular viability after 24 h of treatment with the PAOPA, POEGMA or STL-POEGMA microgel treatments compared to the untreated control cells (Figure 10). Though there was a concentration dependent decrease in cell viability with increasing treatment concentrations, this was only a 10-15% decrease at exceptionally high treatment concentrations (i.e. 2 mg/mL).



*Figure 10.* SHS 5Y Neuronal cell viability following 24 hr exposure to either PAOPA, POEGMA or STL-POEGMA microgels and incubation at  $37^{\circ}C$  (n = 4/treatment)

#### 4.5. Nanoparticle characterization, concentration and lectin conjugation density

The size of all nanoparticle formulations was measured to ensure that the nanoparticles are 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 3). There were no significant differences in particle size as a function of particle STL-functionalization (Table 3). The polydispersity index (PDI) from DLS ranged between 0.07~0.22, suggesting a reasonably narrow distribution of particle sizes, and the TEM showed a spherical particle morphology (Figure 11). Nanoparticle tracking analysis indicated a nanoparticle concentration of 2-4 x 10<sup>13</sup> particles/mL per nanoparticle preparation. All measures of zeta potentials ranged between -11 to -16 mV, with the charge originating from residual sodium cholate at the particle interface following the emulsion process and the net negative charge on the lectin targeting ligand; however, STL

functionalization did not have a significant effect on surface charge (t=7.07, d.f.=2, P=0.5594). The BCA assay showed that an STL conjugation efficiency of 94.0  $\pm$  0.8% was achieved, resulting in a surface density of STL on the nanoparticles of 14.9  $\pm$  5.7 STL/nanoparticle. The higher molecular weight of the Mal-PEG component (M<sub>w</sub> = 3400 D) was chosen to ensure that the maleimide function protruded from the MePEG surface to promote high yield lectin conjugation.



*Figure 11*. TEM images of the haloperidol-loaded PEG-PLGA nanoparticles at 150,000x magnification (a) functionalized nanoparticles (b) non-functionalized nanoparticles

Table 3. Nanoparticle size, polydispersity index and zeta potential for each of the

nanoparticle samples prepared in this study.

Nanoparticle Type and Drug Encapsulated	Nanoparticle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
Empty Nanoparticles	114 <u>+</u> 22	0.139	-14.8 <u>+</u> 0.1
STL-Functionalized	121 <u>+</u> 22	0.221	-16.8 <u>+</u> 0.1
Empty Nanoparticles			
Haloperidol Loaded	121 <u>+</u> 17	0.149	-11.1 <u>+</u> 0.1

Nanoparticles			
STL-Functionalized	132 <u>+</u> 20	0.174	-14.4 <u>+</u> 0.1
Haloperidol Loaded			
Nanoparticles			

## 4.6. Drug quantification and release from functionalized and non-functionalized nanoparticles

The encapsulation efficiency was measured to determine the ratio of the weight of drug incorporated into the nanoparticles 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 high DLCs and EEs, which demonstrates the high efficacy of the nanoparticle preparation process described in the current study. Nanoparticle functionalization was found to have a significant effect on EE and DLC (t=5.81, d.f.=4, P<0.05). The significant decrease in EE for both drug loaded nanoparticles following STL functionalization indicates the loss of some nanoparticles during the additional steps involved in the STL functionalization process. However, no significant drug leaching appears to occur from the nanoparticles that are retained during the STL functionalization process (Table 3).

*Table 4*. Comparison of nanoparticle concentration, encapsulation efficiency, drug loading capacity and cumulative release *in vitro* for each of the nanoparticle samples prepared.

Nanoparticle Type	Nanoparticle	Encapsulation	Drug Loading Capacity
and Drug	Concentration	Efficiency	
Encapsulated	(particles/mL)		

Haloperidol Loaded Nanoparticles	4.89 x 10 <sup>13</sup>	82.5 ± 1.4% *	$0.96 \pm 0.02\%$ *
Functionalized Haloperidol Loaded Nanoparticles	$4.10 \ge 10^{13}$	73.2 ± 0.8% *	0.85 ± 0.01%*

\* = statistical significance of p<0.05 between functionalization and encapsulation efficiency

#### 4.7. In vitro drug release study from STL-NPs and non-functionalized nanoparticles

The *in vitro* release of each drug from the nanoparticles was measured over 96 hours to ensure efficient transport of drug to the targeted brain tissue without premature drug loss. As demonstrated in Figure 12, the total observed *in vitro* release for haloperidol in pH 4.5 acetate buffer and pH 7.4 PBS+0.1% tween at 37 °C was between 5-7% of loaded drug from both the STL-nanoparticles and the non-functionalized nanoparticles after a 96 hr incubation period. This result suggests that minimal drug is lost from the nanoparticles during the period over which they would be transported to the target tissue.



*Figure 12. In vitro* leakage of haloperidol from NP and STL-NP in pH 4.5 acetate buffer (n = 3).

# 4.8. The effect of distance on spray surface area using a single-dose metered nasal spray device

This experiment was performed in order to ensure that when the nanoparticle drug formulation is administered intranasally, the spray surface area would be within the average olfactory epithelium surface area ( $675.2\pm43 \text{ mm}^2$ ) to maximize olfactory epithelial cell targeting (Gross et al., 1982). There was no significant between subjects effect of drug formulation type on spray surface area ( $F_{(2, 54)} = 1.74$ , p< 0.05, p = 0.185). There was also a significant between treatments effect of distance on nasal spray surface area ( $F_{(1, 54)} = 130.08$ , p< 0.0001). Although the 2x2 between subjects ANOVA showed there was no interaction between drug formulation type and distance from the paper, both appear to increase the spray surface area (see Figure 15).



*Figure 13*. The nasal spray device design: a) a design of the nozzle (left) and b) the device viewed from the side displaying an internal view (right).

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*Figure 14.* The nasal spray device design: a) viewed from the top displaying an external view (top) and the internal structure (bottom).



*Figure 15.* Average spray surface area following aerosolization of three different drug solution types (Water, PEG-PLGA nanoparticles (NPs), PEG-PLGA NPs plus Methylene Blue) at two different distances from the vertical surface

## **4.9.** Catalepsy testing following intranasal administration of haloperidol-loaded nanoparticles

Intranasally administered haloperidol loaded STL-functionalized nanoparticles were assessed in terms of their ability to induce catalepsy in rats. Figure 16 shows that there was a significant difference in cataleptic response between the empty nanoparticles and the loaded nanoparticles regardless of route of administration (ANOVA:  $F_{(2, 14)} =$ 81.99, p< 0.0001). Figure 16 also shows that neither the presence of anaesthesia or the type of intranasal administration (i.e. pipette vs. nasal spray device) resulted in any significant difference in cataleptic response though all rats were highly cataleptic (ANOVA:  $F_{(2, 15)} = 0.9925$ , p> 0.05). None of the 3 treatments were found to cause any form of inflammation (ex. irritation or bleeding of the nasal cavity) or any other kind of visible systemic neurotoxic effect (ex. seizures, respiratory failure, etc.).



*Figure 16.* Cataleptic response rating following drug administration based upon the drug treatment received.

## **4.10.** Tissue analysis following intranasal administration of haloperidol-loaded nanoparticles

The concentration of intranasally administered haloperidol-loaded STL functionalized nanoparticles, with and without the use of anaesthesia or the use of a pipette or intranasal spray device was measured within the striatum and olfactory bulb. Figure 17 shows that there was a significant difference in the haloperidol concentration within the striatum between the empty nanoparticles and the loaded nanoparticles regardless of route of administration (ANOVA:  $F_{(2, 13)} = 170.6$ , p< 0.0001). Figure 17 also shows a significant difference in the haloperidol concentration achieved within the olfactory bulb between the non-anaesthetized rats given STL-funcationalized haloperidolloaded nanoparticles and anaesthetized STL-funcationalized haloperidol-loaded nanoparticles administered intranasally via pipette versus the intranasal spray device (ANOVA:  $F_{(2,15)} = 17.52$ , p< 0.001). However, there was no significant difference in the haloperidol concentration within the olfactory bulb between non-anaesthetized and anaesthetized rats given haloperidol-loaded STL-nanoparticles intranasally with a pipette though this was much simpler to do under anaesthesia (ANOVA:  $F_{(2, 15)} = 17.52$ , p> 0.05). The anaesthetized rats given haloperidol-loaded STL-nanoparticles also showed a significantly higher haloperidol concentration within the olfactory bulb after intranasal administration using the nasal spray device than those administered using a pipette (ANOVA:  $F_{(2,15)} = 17.52$ , p< 0.01). Analogously, figure 18 shows that there was a significant difference in the haloperidol concentration within the striatum between the empty nanoparticles and the loaded nanoparticles regardless of route of administration (ANOVA: F  $_{(2, 13)}$  = 557.6, p< 0.0001). Figure 18 also shows that there was a significant difference in the haloperidol concentration achieved within the striatum between the nonanaesthetized rats given STL-funcationalized haloperidol-loaded nanoparticles via pipette and anaesthetized STL-funcationalized haloperidol-loaded nanoparticles administered intranasally using the intranasal spray device (ANOVA:  $F_{(2, 15)} = 8.729$ , p< 0.01). However, there was no significant difference in the haloperidol concentration within the olfactory bulb between non-anaesthetized and anaesthetized rats given haloperidol-loaded STL-nanoparticles intranasally with a pipette (ANOVA:  $F_{(2, 15)} = 8.729$ , p> 0.05). The anaesthetized rats given haloperidol-loaded STL-nanoparticles also showed a significantly higher haloperidol concentration within the striatum after intranasal administration using the nasal spray device than those administered using a pipette (ANOVA:  $F_{(2, 15)} = 8.729$ , p< 0.05), further indicating the efficacy of the use of an intranasal spray device for intranasal administration studies.



Administration and Formulation Type

*Figure 17*. Olfactory bulb tissue concentrations of haloperidol following drug administration based upon the drug treatment received: empty nanoparticles administered intranasally using anaesthesia using a pipette (Empty NPs (IN-P + A)), haloperidol STLnanoparticles administered intranasally without anaesthesia using a pipette (HP-STL-NPs (IN)), haloperidol STL-nanoparticles administered IN with anaesthesia using a pipette (HP-STL-NPs (IN-P+ A)) and haloperidol STL-nanoparticles administered IN with anaesthesia using a nasal spray device (HP-STL-NPs (IN-D+ A)).



*Figure 18.* Striatum tissue concentrations of haloperidol following drug administration based upon the drug treatment received: empty nanoparticles administered intranasally using anaesthesia using a pipette (Empty NPs (IN-P + A)), haloperidol STL-nanoparticles administered intranasally without anaesthesia using a pipette (HP-STL-NPs (IN)), haloperidol STL-nanoparticles administered IN with anaesthesia using a pipette (HP-STL-NPs (IN-P+ A)) and haloperidol STL-nanoparticles administered IN with anaesthesia using a nasal spray device (HP-STL-NPs (IN-D+ A)).

### **4.11.** Tissue and blood serum analysis following intranasal administration of rhodamine-labelled POEGMA microgels

The microgel sizes, PDIs and particle concentrations were determined as that of the other POEGMA microgels (see Table 5). The brain tissue concentrations of IP administered rhodamine-labelled microgels of three different sizes were also measured within the brain (PFC, striatum and cerebellum) and the major organs (liver, lungs, spleen and kidneys.

*Table 5.* Rhodamine Microgel size, polydispersity index and microgel concentration for each of the microgel sizes prepared in this study.

Microgel Type and Drug	Microgel	Polydispersity	Microgel Concentration
Encapsulated	Size (nm)	Index (PDI)	(particles/mL)
Rhodamine labelled	78.8 <u>+</u> 1.59	0.04 <u>+</u> 0.001	$2.78 \times 10^{11}$
Microgels 1			
Rhodamine labelled	122.4 <u>+</u> 2.47	0.026 <u>+</u> 0.001	$3.82 \times 10^{11}$
Microgels 2			
Rhodamine labelled	219.9 <u>+</u> 4.44	0.105 <u>+</u> 0.002	$3.66 \times 10^{11}$
Microgels 3			

Figure 19 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 comparisions 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 rhodaminelabelled 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 rhodaminelabelled microgel size (ANOVA: F  $_{(3, 44)}$  = 28.70, p< 0.0001). Figure 19 may shows a trend in that the administration of smaller microgels results in greater brain tissue uptake in all three regions.



*Figure 19.* Brain tissue concentrations of rhodamine-labelled microgels following intraperitoneal (IP) injection at 10 mg/kg of varying microgel sizes with or without a rhodamine label (n=5): prefrontal cortex (PFC), striatum and cerebellum

Figure 20 shows that all between groups comparisions 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 20 may suggest a trend in that the administration of smaller microgels results in greater major organ tissue uptake in all four organs.



*Figure 20.* Major Organ tissue concentrations of rhodamine-labelled microgels following intraperitoneal (IP) injection at 10 mg/kg of varying microgel sizes with or without a rhodamine label (n=4): liver, lungs, spleen and kidneys

Figure 21 shows that there was no significant difference in rhodamine-labelled microgel total blood serum concentration (ANOVA:  $F_{(2, 36)} = 1.07$ , p>0.05, p = 0.4006), but there was a significant difference between the non-labelled microgels and the rhodamine-labelled microgels regardless of microgel size (ANOVA:  $F_{(3, 8)} = 7.368$ , p< 0.05). None of the between groups comparisons between any of the microgel sizes were significant, which may have been the result of nanoprecipitation in the anti-coagulant.



*Figure 21.* Blood Serum concentrations of rhodamine-labelled microgels following intraperitoneal (IP) injection at 10 mg/kg of varying microgel sizes with or without a rhodamine label (n=3)

#### **5 DISCUSSION**

Based on the results presented, STL-functionalized microgel and nanoparticle formulations provide three key advantages for effective APD administration. First, this is the first demonstration of not only an APD-loaded POEGMA microgel formulation, but one to be functionalized for the purposes of olfactory epithelial cell-specific targeting for intranasal administration that could significantly improve the neural biodistribution of hydrophilic drugs. Second, the use of a single-dose intranasal spray device for the atomization of APD loaded formulations over that of the traditionally used pipette resulting in a greater amount of drug reaching the brain via the intranasal route. Third, this is the first presentation of a POEGMA microgel formulation crossing the BBB and to be tracked *in vivo* following administration. Fourth, though rhodamine-labelled POEGMA microgels of smaller sizes reached the brain and were taken up by major organs in greater concentrations compared than the larger microgels, microgels of sizes >150 nm were still able to pass the BBB contrary to the prior literature (Chhajed, Sangale, & Barhate, 2011).

### **5.1 POEGMA microgel and PEG-PLGA nanoparticle characterization,** concentration and lectin conjugation density

Vila *et al.*, 2005 showed that a PEG-PLA particle size of <200 nm was directly related to the efficiency of <sup>125</sup>I-tetanus toxoid encapsulated nanoparticle transport across the nasal mucosa via cellular and tissue uptake. Small particle size (<150 nm) is necessary 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 (Veiseh, Gunn, & Zhang, 2010). The formulations reported herein for PAOPA and haloperidol (non-functionalized and STL-functionalized) were all <150 nm in size, which is generally smaller than that of other reported APD-loaded nanoparticle preparations from the literature (Benvegnú *et al.*, 2011; Budhian, Siegel, & Winey, 2008; Muthu & Singh, 2007; Muthu & Singh, 2008).

There have been several studies analyzing the sizes of drug-loaded microgels or microgels administer drugs though few have specifically analyzed size (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 hydrogels through selective sulfhydryl groups engineered on the CaM molecule presenting a volume of 1 mm<sup>3</sup> (Murphy *et al.*, 2007). In another study, N-[(2-hydroxy-3-trimethylammonium) propyl] chitosanchloride (HTCC)-PEG- $\alpha$ - $\beta$ -glycerophosphate ( $\alpha$ - $\beta$ -GP) hydrogels prepared for intranasal administration, yet this was too large for effective intransal transport as the size of the chitosan derivative was 600-1000 KDa (Wu *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 cleavage without any drug loading (He *et al.*, 2009). Outside of this, 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). 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). This was contrary to what occurred in the POEGMA microgels with PAOPA in which the addition of PAOPA, where the sizes of the gels slightly decreased with the addition of PAOPA perhaps due to the intermolecular forces between that of PAOPA and the internal acrylic acid residues present in the microgels preventing swelling due to water influx. The reason for the small microgel sizes achieved can also potentially be attributed to the relative amount of SDS added during the initial POEGMA synthesis (Smeets & Hoare, 2013)

The exception to the sizes reported for the haloperidol-loaded nanoparticles on the other hand is the work of Hans *et al.* (2005), who developed haloperidol loaded nanoparticles smaller ( $49.7\pm4.3$  nm) than those prepared in this study but required covalent conjugation of haloperidol to the terminal carboxylic acid of the PLA polymer. Though effective in creating small particle sizes, the Hans *et al.* method used requires a 20:1 w/w polymer to drug ratio to achieve a conjugation efficiency of  $64.8\pm21\%$  prior to the emulsion process, which may not be very cost effective and significantly limits the dose of drug that can be safely administered *in vivo*.

The particle design for both formulations used 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 and the nanoparticles displayed only a relatively small 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 after one week of storage, with any associated particles easily redispersed by simple shaking; this particle stability can be regarded as favorable for nasal epithelial as well as endothelial cell transport (Gao *et al.*, 2006; Muthu & Singh, 2008).

## 5.2 Drug quantification and release from functionalized and non-functionalized drug carriers

Though there has never been an APD encapsulated into a microgel formulation, there have been several hydrophilic drugs entrapped in microgels (Rao *et al.*, 2006). 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 absorptions or encapsulations 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). The strong association leading to the moderate PAOPA encapsulations can be attributed to the hydrogen bonding interactions between the –COOH-terminal of the acrylic acid residues and the amide groups present in PAOPA.

The current study also showed high EEs (73-85%) that are comparable to or greater than those reported previously with haloperidol-loaded nanoparticles (Benvegnú *et al.*, 2011; Budhian, Siegel, & Winey, 2005; Budhian, Siegel, & Winey, 2008; Hans *et al.*, 2005). These findings can be attributed to the polymer-drug w/w ratio (7:1) used in the emulsion process and hydrogen bonding interactions between the –COOH-terminal end group on PLGA and the ketone group in haloperidol, which has previously been shown to increase haloperidol EE by 30% relative to preparations with a terminal PLGA methoxy group (Budhian, Siegel, & Winey, 2005). The use of a neutral PEG nanoparticle coating as opposed to a cationic chitosan coating that would electrostatically repel haloperidol at physiological pH may also contribute to this high observed drug loading; indeed, nanoparticles with chitosan coatings have shown lower haloperidol encapsulation efficiencies than PEG coated nanoparticles (Budhian, Siegel, & Winey, 2005; Hans *et al.*, 2005).

Drug encapsulation measurements can also be strongly influenced by the method used to perform the measurements. Previous studies obtained similar or lower EEs by encapsulating free haloperidol with haloperidol conjugated PEG-PLA polymers, measuring encapsulation after d-DMSO dissolution of the nanoparticle via proton nuclear magnetic resonance (<sup>1</sup>H NMR) and/or using interfacial polymer deposition to measure

free haloperidol in the ultrafiltrate (Benvegnú *et al.*, 2011; Hans *et al.*, 2005). We quantified the amount of drug lost through every step of the particle formation and functionalization processes with both drug carriers, which was then subtracted from the total amount of drug initially added to determine encapsulation. Previous papers have reported high encapsulation efficiencies using a similar method for quantifying drug loss during the emulsion process, but may not have accounted for drug loss during the transfer of solutions between steps (Benvegnú *et al.*, 2011).

## 5.3 *In vitro* drug release study from STL-functionalized and non-functionalized PAOPA-loaded POEGMA microgels

The rate of *in vitro* release of the loaded amount of PAOPA in physiological conditions (pH 7.4 at  $37^{\circ}$ C) is fairly slow though it leads to nearly 100% release of the drug over 96 hours (demonstrating minimal drug leakage and the potential for efficient drug transport to its allosteric molecular target, the dopamine D2 receptor). This release profile would be due to the slow partitioning of PAOPA from the microgel complex (log*P* value of PAOPA = -1.314), which may be improved via the placing the PAOPA-loaded microgels into a PAOPA solution resulting in equilibrium. The strong drug-polymer interaction between haloperidol and the hydrophobic PLGA block (log*P* value of haloperidol = 3.36) may explain the low release rate (3-4%) of haloperidol observed in the endo-lysosomal medium (pH 4.5) compared to prior literature over 5 days (Benvegnú *et al.*, 2011; Budhian, Siegel, & Winey, 2005; Chen *et al.*, 2011; Corrigan & Li, 2009; Panyam *et al.*, 2003; Patel *et al.*, 2011). This observed lack of quick release suggests that

most of the PAOPA or haloperidol 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 this formulation in retaining the encapsulated drug is integral to minimize drug leakage from the microgels or nanoparticles before they reach the desired target of interest (in our case the dopamine D2 receptor) and may allow for the drug stay in the system longer than the 6 hr half-life of PAOPA therby improving the steady state level (20 mL/min/kg) following IV administration (Tan *et al.*, 2013).

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

None of the treatments (PAOPA, POEGMA, or the STL-POEGMA microgels) were found to have any detrimental effect on cellular viability in either cell line. Studies have been able to show at concentrations up to 1mg/ml, POEGMA polymers are not toxic to human HeLa cervical or MCF-7 breast cancer cells following 24 hr treatment (Cheng *et al.*, 2011). Though not using POEGMA specifically, another group was able to show that Zwitterionic Poly(Sulfobetaine Methacrylate) polymers were non-toxic to Bovine Aortic Endothelial Cells (BAEC) cells following 24 hr treatment (Lalani & Liu, 2012). 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. This demonstrates that this formulation won't 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.

## 5.5 Catalepsy testing following intranasal administration of haloperidol-loaded nanoparticles using a single-dose metered nasal spray device

Due to the spray surface area being smaller than that of the surface area of the rat olfactory epithelium (<675.2+43 mm<sup>2</sup>), the nasal spray device was effective in intranasally administering haloperidol-loaded STL-functionalized nanoparticles (Gross et al., 1982). When the haloperidol-loaded nanoparticles were administered to naive rats, the haloperidol nanoparticle formulations successfully induced catalepsy regardless of the mode of administration. Administration of STL-functionalized nanoparticles intranasally via pipette was just as effective at inducing catalepsy in naive rats or using the nasal spray device (Figure 4), offering an equally effective but significantly less invasive method of local drug delivery to the brain. All rats receiving haloperidol with a nanoparticle formulation were highly cataleptic, with no difference in catalepsy rating observed. This lack of difference in catalepsy responses may be due to the fact that haloperidol doses as low as 0.25 mg/kg have been shown to induce catalepsy (Öhman *et al.*, 1977). However, the results clearly demonstrate that these nanoparticle formulations are capable of transporting haloperidol across the BBB to their target (the dopamine D2 receptor) and that the less invasive intranasal route of administration using an intranasal spray device is just as effective as other routes of administration or the use of a pipette.

## 5.6 Tissue analysis following intranasal administration of haloperidol-loaded nanoparticles

In order to provide evidence for the superiority of using an intranasal spray device over that of a pipette with or without anaesthesia in the intranasal administration of STL-

functionalized nanoparticles at passing the BBB, region-specific haloperidol concentrations were analyzed. The regions chosen were the striatum, which contains one of the highest D2 receptor concentrations within the brain, and the olfactory bulb, which has previously been shown to contain the highest nanoparticle concentration of drug within the olfactory bulb 1 hour following intranasal administration (Chen et al., 2011; Weiner & Brann, 1989). The concentration of haloperidol was significantly higher within both brain regions following intranasal administration of STL-functionalized haloperidolloaded nanoparticles using the intranasal spray device following anaesthesia, suggesting that not only does STL nanoparticle surface functionalization significantly increase the efficacy of particle transport across the nasal epithelium, but the aerosolization of the particles using a nasal spray device significantly improves drug carrier delivery and thus facilitates greater amounts of haloperidol reaching the striatum. In contrast, unanaesthetized and anaesthetized rats intranasally administered haloperidol-loaded nanoparticles using a pipette showed no significant differences in haloperidol concentration using a pipette. Though intranasal administration to anaesthetized rodents was far simpler, the rate at which the droplets of formulation reached the olfactory epithelium was not different and explains why no difference in tissue concentration was observed. All of the anaesthetized rats receiving intranasally administered haloperidolloaded nanoparticles using the intranasal spray device demonstrated tissue concentrations of haloperidol 3-3.5 fold greater than that of previously reported striatal concentrations of haloperidol alone following IP injection (Igarashi et al., 1995; Öhman et al., 1977). This result provides *in vivo* evidence that nasal spray device aerosolized haloperidol-loaded

nanoparticles administered intranasally facilitate greater haloperidol uptake into the brain over that of non-aerosolized formulations and that of injectable routes of administration.

### 5.7 Tissue and blood analysis following intraperitoneal administration of rhodamine-labelled POEGMA microgels

Before attempting to administer POAPA-loaded microgels using any mode of administration, it was necessary to determine whether the POEGMA microgels were capable of passing the BBB following intranasal administration as this has never been 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 an 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). This is perhaps due to the soft, flexible and relatively uncharged character of these microgels, allowing it 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-seperated 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. Though the microgels were detected in blood serum following blood extraction, it appears that 90% of the microgels are precipitated by the anti-coagulant with the blood cells and that particle size has no bearing on blood microgel concentrations (unreported data). Microgels such as those made from POEGMA have been demonstrated to be pH-sensitive, resulting in precipitation due to a decrease in the lower critical solution temperature (LCST) (Smeets & Hoare, 2013). From this it could be postulated that the greatest tissue microgel concentrations would potentially be found in

the small or large intestine at the site of injection following administration. This aside, the brain tissue concentrations are very promising in that it proves that POEGMA microgels can effectively pass the BBB and that if these microgels were to be used for the targeting of other major organs, they would also be successful in ensuring that the encapsulated hydrophilic drug reaches its target of interest.

#### **5.8 Future directions**

The next step in this line of research should be to determine whether the intranasal administration of rhodamine-labelled microgels leads to higher brain and major organ tissue concentrations than that of intraperitoneally administered rhodamine-labelled microgels. We would expect the intranasal route of administration to lead to higher brain and major organ tissue concentrations of rhodamine-labelled microgels based upon previous work with DiR-loaded PEG-PLGA emulsions administered to naive rats (Chen et al., 2011). From here there would need to be an attempt to determine whether the PAOPA-loaded STL-functionalized POEGMA microgels are effective in reversing schizophrenic symptoms in a rodent model using several routes of administration such as oral, intravenous (or intraperitoneal), and intranasal routes in comparison to that observed with PAOPA in absence of a drug carrier (Tan *et al.*, 2013). The phenylcyclidine (PCP) and amphetamine models have shown to be a excellent model of schizophrenic behavior in rodent and human models due to many of the symptoms of extended PCP use resembling that of those observed in schizophrenic patients (i.e. in the positive symptoms, negative symptoms, and cognitive dysfunction) (Beyaert et al., 2013; Jentsch & Roth, 1999). Behavioral attenuation of schizophrenic symptomatology and PAOPA brain tissue
concentration analysis using multiple routes of administration will thus be integral in furthering the progress described here. We have shown that lectin-functionalized PEG-PLGA nanoparticles are highly effective intranasal delivery vehicles for haloperidol, demonstrating that the STL-functionalizated drug carriers and intranasal route could potentially be very successful for the treatment of schizophrenia with PAOPA. In future studies, it may also be interesting to compare the cellular uptake and brain tissues concentrations of different nanoparticle formulation types (i.e. POEGMA microgels vs. SLNs vs. PEG-PLGA polymersomes) designed for hydrophobic or hydrophilic drugs using several routes of administration (compared to the intranasal route in particular). The success of one of these drug carrier formulations over another may be based upon the size, surface charge and drug loading characteristics of several different antipsychotics described in the prior literature. This comparison could determine which nanoparticle or microgel formulation would most significantly improve antipsychotic therapeutic efficacy using an intranasal route of administration.

## **6 CONCLUSIONS**

In conclusion, this is the first time an APD has been encapsulated within a POEGMA drug carrier and functionalized for the purposes of intranasal route-specific cellular targeting of olfactory nasal epithelial cells using STL surface functionalization. The results of this study also show that the lectin-targeted nanoparticle formulations developed coupled with a single-dose nasal spray device for intranasal administration allow for a greater amount of drug to enter the brain via an intranasal route of

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administration. The current study is also the first to show that POEGMA microgels are capable of crossing the BBB via the IP route of administration, paving the way for POEGMA microgels to be loaded with APDs (including other APDs) for testing with additional routes of administration (i.e. the intranasal route of administration). Consequently, these formulations (i.e. including PAOPA-loaded microgels) have the potential to create an effective therapeutic response at a lower dose than unencapsulated CNS therapeutics. Furthermore, the higher efficacy of intranasal administration achieved with these particles (particularly with the lectin-functionalized particles) compared to more conventional IP administrations provides a far less invasive method of drug administration than that of IP injection, giving practitioners as well as patients more flexibility in choosing drug treatment.

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