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Polymeric nanoparticles for efficient nose-to-brain delivery

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Brain disorders affect over one billion people globally, presenting significant challenges for effective treatment due to the limited drug bioavailability in the brain. This issue is largely attributed to the presence of the blood-brain barrier (BBB), a highly selective and restrictive biological barrier. Nose-to-brain delivery (NtBD) *via* intranasal administration has emerged as a compelling and non-invasive strategy to bypass the BBB, leveraging the anatomical and physiological characteristics of the nasal cavity to enable direct drug transport to the brain. Among the various delivery strategies, nanoparticles, and polymeric nanoparticles in particular, stand out due to their growing attention, offering biocompatibility, biodegradability, and customizable designs. This review explores the key physicochemical characteristics of polymeric nanoparticles, including size, charge, and surface modifications, and analyses their impact on mucosal adhesion, mucopenetration, and brain targeting efficiency by crossing different biological barriers. Functionalization strategies, such as mucoadhesive coatings, cell-penetrating peptides, and targeting ligands, are discussed comprehensively to enhance drug stability, residence time, and cellular uptake. Evaluation techniques covering *in vitro*, *ex vivo*, and *in vivo* models are critically reviewed, emphasizing their relevance for elucidating transport mechanisms and assessing the therapeutic potential of nanoparticles. Special focus is given to the applications of polymeric nanoparticles in treating several brain diseases, where they show promising potential in optimizing drug delivery efficiency and therapeutic outcomes. By synthesizing current advancements, this review offers a robust framework for the rational development of next-generation polymeric nanoparticles tailored to advanced NtBD systems.

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1. Introduction

Brain disorders affect over one billion people all over the world[1] with a huge impact on both patients and caregivers,

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but there is still a lack of effective treatments for numerous diseases.[2] This lack of efficiency is partly due to the low brain bioavailability of the drug after oral or intravenous (IV) administration because of multiple physiological barriers.[3] For example, to treat Alzheimer's disease (AD), there are only four drugs available and they only slow down the natural course of the disease. This lack of effective drugs is mainly due to the difficulty to treat the disease but also related to poor brain bioavailability of the candidates.[4]

Among these biological barriers, the blood-brain barrier (BBB), which acts as a barrier around blood vessels in the brain, is the most difficult to cross due to very selective tight junctions.[5] In addition to the BBB, others barriers are also present in the brain, such as the blood-cerebrospinal fluid barrier and the arachnoid barrier. The superposition of these barriers decreases the possibility of drug delivery from the blood to the cerebrospinal fluid (CSF) and the subarachnoid space.[6] Therefore, non-invasive strategies for barrier avoidance have been developed to improve brain bioavailability. A promising strategy is the intranasal administration (INA). Due to the anatomy of the nasal cavity and the presence of neuronal cilia directly in the nasal cavity, INA offers a direct passage to the brain. This avoids the BBB as well as other disadvantages of both oral and IV administration, the main one being the first hepatic passage.[7,8] To date, over 300 clinical trials are ongoing to test INA for the delivery of drugs against brain disorders.

Despite the emergence of nasal drug delivery systems to treat brain disorders, improving the efficacy of brain delivery is still a major consideration. Regardless of the many advantages of nose-to-brain delivery (NtBD), there are several factors to be considered when designing an intranasally delivered drug: (i) numerous enzymes can cause degradation, (ii) mucociliary clearance can reduce the time of contact with the nasal mucosa, and (iii) the characteristics, such as hydrophobicity, molecular weight, and ionisation, can affect its adsorption through the nasal mucosa.[9–11]

Drug carriers, such as nanoparticles (NPs), have been developed for several years for their numerous applications, from vectorizing many therapeutical agents or as imaging tools, especially in cancerology[12,13] but also in various fields such as enamel caries treatments.[14] They have been designed to both protect the drug and improve its passage through various biological barriers, particularly in the context of NtBD.[9,11] NPs are a wide class of drug delivery systems facilitating the transport of drugs across the mucosa.[3] NPs have numerous advantages including drug protection, higher bioavailability by increasing the residence time on the nasal mucosa, possibility of surface modifications, allowance of a higher drug concentration in the brain but also maintenance of the therapeutic effect of the drug.[15] They mainly consist of polymeric NPs, lipid-based NPs, liposomes, nanoemulsions, and nanogels. Among these NPs, the most studied ones for NtBD are inorganic NPs, lipid-based NPs, and polymeric NPs.

Inorganic NPs are often composed of silver, gold, or iron oxide. These very small NPs, from 40 nm to 80 nm, can be functionalized and used as contrast agents for different imaging systems and are largely studied for cancer therapies.[12] Despite their benefits for NtBD, their use is questioned due to their lack of biocompatibility and their toxicity.[16] Lipid-based NPs have also been largely studied for NtBD, as the lipid composition can vary, and therefore can provide different benefits that can be of interest in this context. The use of cationic lipids, for example, allows a prolonged residency time in the nasal cavity. Thanks to their permanent positive charge, they can interact with the mucus layer, providing some mucoadhesive properties. Ionizable lipids can be protonated at low pH and neutral at physiological pH and can also be of use in the context of NtBD. All lipids composing the NPs can be chosen

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Dr Claire Monge obtained a Ph. D. of Physiology and Pharmacology from the Université Grenoble Alpes (Grenoble, France). After graduating, she worked as a postdoc at Radboud University (Nijmegen, The Netherlands) on cell bioenergetics. Back to France, she carried a 4-year project on the development of biomaterials for muscle tissue engineering. She is now a permanent research scientist at CNRS (French National Center for Scientific Research) at the LBTI in Lyon, France. She leads the NanoVec lab and carries out projects on the development of nanovectors for vaccine and biotherapy delivery at mucosal surfaces.

Sophie Richard

Dr Sophie Richard is currently an Associate Professor of Cell Biology at the Claude Bernard University, Lyon, France. After graduating as a biotechnology engineer, she received her PhD. in 2015 in Biomedical Engineering from Paris Nord University, focusing on nanomedicine, imaging, and therapy for brain cancers. She then pursued a postdoctoral fellowship in regenerative medicine, focusing on nanoparticle-based therapies, and also contributed to projects involving molecular targeting strategies. Her current research focuses on the use of nanoparticles to enhance mucosal penetration for targeted therapies and the development of imaging techniques to study their biological fate and biodistribution in vivo.

Table 1 Summary of polymeric NPs composition, characteristics and evaluation for NtBD

Polymer used	Other molecules	Drug adsorbed	Synthesis method	Size (nm)	PDI	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)	NtB *in vitro* evaluation	NtB *ex vivo* evaluation	NtB *in vivo* evaluation	Application	Ref.
Alginate	Sodium dodecylbenzenesulfonate	Serotonin or 5-hydroxytryptophan	Gelation	220 ± 4					Cellular uptake			Depression	232
Chitosan		Ritigotine	Ionic gelation	75.37 ± 3.37	0.368 ± 0.02	25.53 ± 0.45		96.08 ± 0.01	Cellular uptake Neuroprotective and antioxidant activity		Pharmacokinetic and pharmacodynamic studies	Parkinson's disease	58
Chitosan		Pramipexole dihydrochloride	Ionic gelation	292.5 ± 8.8		14.0 ± 2.89		91.25 ± 0.95	Drug diffusion	Franz diffusion cell	Pharmacodynamic studies	Parkinson's disease	166
Chitosan	Tween 80	Leucine-enkephalin	Ionic gelation	443 ± 23	0.317 ± 0.17	15 ± 2	14 ± 1.3	78.28 ± 3.8	Release study Mucoadhesion study	Franz diffusion cell	Pharmacokinetic and pharmacodynamic studies	Pain	177
Chitosan	Glutaraldehyde Human serum albumin	Sulforhodamine B	Desolvation-coacervation	267 ± 6	0.12 ± 0.02	44 ± 1		63 ± 1	Release study Cellular uptake Permeation across cells Effect on cell junctions	Franz diffusion cell			176
Chitosan		Bromocriptine	Ionic gelation	161.3 ± 4.7	0.44 ± 0.03	40.32 ± 2.78	37.8 ± 1.8	84.2 ± 3.5			Pharmacokinetic and pharmacodynamic studies	Parkinson's disease	219
Chitosan		Rutin	Ionic gelation	92.28 ± 2.96	0.206 ± 0.006	31.04 ± 1.91	39.48 ± 3.16	84.98 ± 4.18	Release study Mucoadhesion study	Franz diffusion cell	Pharmacokinetic and pharmacodynamic studies	Cerebral ischemia/reperfusion injury	221
Chitosan		Dopamine	Ionic gelation	372 ± 81	0.26 ± 0.07	9.3 ± 1.3		54.5 ± 0.7	Release study		Pharmacokinetic studies	Parkinson's disease	233
Chitosan		Buspirone hydrochloride	Ionic gelation	226.7 ± 2.52	0.483 ± 0.031		49.67 ± 5.5	81.13 ± 2.8	Release study	Franz diffusion cell Bioadhesion	Pharmacokinetic and pharmacodynamic studies	General anxiety disorder	181
Chitosan		Ropinirole hydrochloride	Ionic gelation	173.7 ± 2.32	0.39 ± 0.03	32.7 ± 1.5	13.8 ± 1.7	69.6 ± 3.3	Release study	Franz diffusion cell	Pharmacokinetic studies	Parkinson's disease	178
Chitosan		Quetiapine fumarate	Ionic gelation	131.08 ± 7.45	0.252 ± 0.064	34.4 ± 1.87		89.93 ± 3.85		Franz diffusion cell Histopathology	Pharmacokinetic studies	Schizophrenia	167
Chitosan	Maisine Labrafac Lecithin	Simvastatin	Self-assembly	204.5 ± 15.4	0.098 ± 0.040	48.45 ± 4.09		98.52 ± 1.33	Release study		Pharmacokinetic studies	Alzheimer's disease	192
Chitosan		Midazolam	Ionic gelation	241.2 ± 12.25	0.389 ± 0.056		36.45 ± 2.14	88.68 ± 1.22	Release study	Franz diffusion cell	Pharmacokinetic studies	Seizure	168
Chitosan		Rasagiline	Ionic gelation	151.1 ± 10.31	0.380 ± 0.01			96.43 ± 4.23	Release study	Franz diffusion cell	Pharmacokinetic studies	Parkinson's disease	20
Chitosan	Poloxamer 407 Lecithin	Piribedil	Solvent injection	147.5 ± 7.9	0.291 ± 0.012	18.1 ± 0.6	12.1 ± 0.7	53.5 ± 2.1			Pharmacokinetic studies	Parkinson's disease	60
Chitosan		Rivastigmine	Ionic gelation	185.4 ± 8.4	0.391 ± 0.065	38.4 ± 2.85	43.37 ± 3.9	85.3 ± 3.5	Release study	Franz diffusion cell	Pharmacokinetic studies	Alzheimer's disease	19
Chitosan		Venlafaxine	Ionic gelation	167 ± 6.5	0.367 ± 0.045	23.83 ± 1.76	32.25 ± 1.63	79.3 ± 2.6		Franz diffusion cell	Pharmacokinetic studies	Depression	179
Chitosan		Levodopa	Ionic gelation	164.5	0.248	28.3		56.2	Mucoadhesion study Release study		Pharmacokinetic studies	Parkinson's disease	234

Table 1 *(Contd.)*

Polymer used	Other molecules	Drug adsorbed	Synthesis method	Size (nm)	PDI	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)	NtB *in vitro* evaluation	NtB *ex vivo* evaluation	NtB *in vivo* evaluation	Application	Ref.
Chitosan		Bromocriptine	Ionic gelation	161.3 ± 4.7	0.44 ± 0.03	40.32 ± 2.78	37.8 ± 1.8	84.2 ± 3.5			Nasal clearance study Pharmacokinetic studies	Parkinson's disease	198
Chitosan		Selegiline	Ionic gelation	215 ± 34.71	0.214 ± 0.041	17.06		70 ± 2.71	Mucoadhesion study		Pharmacokinetic and pharmacodynamic studies	Depression	101
Chitosan		Tapentadol hydrochloride	Ionic gelation	201.2 ± 1.5	0.201 ± 0.01	49.3 ± 1.2	17.25 ± 1.38	63.49 ± 1.61	Release study Mucoadhesion study	Franz diffusion cell	Pharmacokinetic and pharmacodynamic studies	Pain	169
Chitosan		Nicardipine	Ionic gelation	439.6 ± 11.9	0.307	21.05 ± 0.48	17.79 ± 4.85		Permeability Release study		Pharmacokinetic studies	Brain oedema	139
Chitosan	PVA		Self-assembly	249 ± 26	0.26 ± 0.01	10 ± 1			Permeation across a RPMI 2650 monolayer cultivated in liquid–liquid or in ALI conditions Cellular uptake				148
Chitosan		siRNA	Ionic gelation	123.6 ± 2.13		47.5 ± 2.68					Pharmacodynamic studies	Huntington's disease	222
Chitosan	Lecithin	Agomelatine	Emulsification	190	0.337	57.8		98.45	Release study Mucoadhesion study		Pharmacokinetic studies	Depression	59
Chitosan		Dopamine	Nanoprecipitation	258 ± 50	0.48	−32.4 ± 1.6		94 ± 3	Release study Cellular uptake			Parkinson's disease	235
Chitosan	Span 80 Tween 80	Iodoisovanillin	Emulsification	141 ± 2	0.23 ± 0.02	−17.4 ± 0.3					Pharmacokinetic studies	Brain tumours	195
Chitosan		β-Galactosidase	Ionic gelation	140 ± 17	0.227 ± 0.045	15.7 ± 1.6		51 ± 4	Cellular uptake				156
Chitosan		Oxcarbazepine	Ionic gelation	189 ± 16.7	0.315 ± 0.01	31.4 ± 2.58		97.5 ± 0.06	Release study		Pharmacodynamic studies	Epilepsy	200
Chitosan	β-Asarone	Astragaloside IV	Ionic gelation	117.8 ± 2.8	0.253 ± 0.013	22.74 ± 3.04	0.14 ± 0.019		Release study Cellular uptake		Pharmacokinetic and pharmacodynamic studies	Multiple sclerosis	152
Chitosan		Centella asiatica	Ionic gelation	210.5	0.260	−14.5 ± 2.43				Franz diffusion cell Nasal ciliotoxicity study		Nerve tonic	164
Chitosan	Okra gum	Esculin	Ionic gelation	312.6 ± 0.4		18.31 ± 3.2		43.24 ± 1.31	Release study Mucoadhesion study	Franz diffusion cell	Pharmacokinetic studies	Neurodegenerative diseases	236
Chitosan		Zolmitriptan	Ionic gelation	216.13 ± 15.71		25.46 ± 1.51		87.38 ± 1.51			Pharmacokinetic and pharmacodynamic studies	Migraine	218
Chitosan		Vinpocetine	Ionic gelation	130.6 ± 8.38	0.125	40.81 ± 0.11	61 ± 0.89	97.56 ± 0.04	Release study		Pharmacokinetic studies	Alzheimer's disease	205
Chitosan		Naringin	Ionic gelation	150			5				Pharmacodynamic studies	Neuroprotection	237

Table 1 *(Contd.)*

Polymer used	Other molecules	Drug adsorbed	Synthesis method	Size (nm)	PDI	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)	NtB *in vitro* evaluation	NtB *ex vivo* evaluation	NtB *in vivo* evaluation	Application	Ref.
Chitosan	Stearic acid Oleic acid Tween 80	Risperidone	Emulsification	132.7		11.8 ± 2	7.6		Release study	Franz diffusion cell	Pharmacokinetic and pharmacodynamic studies	Schizophrenia	55
Chitosan		Galantamine	Ionic gelation	149.2 ± 0.9	0.222 ± 0.005	27.2		88.23 ± 0.69	Mucoadhesion study		Pharmacodynamic studies	Alzheimer's disease	56
Chitosan	Tween 80 Poloxamer 188	Selegiline hydrochloride	Ionic gelation	63.1	0.201	35.2		74.77 ± 0.31	Release study Mucoadhesion study		Pharmacodynamic studies	Parkinson's disease	145
PCL		Carboplatin	Emulsification	311.6 ± 4.7	0.21 ± 0.03	−16.3 ± 3.7		27.95 ± 4.21	Release study	Franz diffusion cell Histopathology	Pharmacokinetic studies	Brain tumours	74
PCL	Poloxamer 188 Poloxamer 407	Aripiprazole	Nanoprecipitation	199.2 ± 5.65		−21.4 ± 4.6		69.2 ± 2.34	Release study	Franz diffusion cell Histopathology	Pharmacokinetic studies	Schizophrenia	73
PCL	PEG	Curcumin	Emulsification	113.3 ± 3.1	0.12 ± 0.03	−14.43 ± 0.25		96.6			Pharmacokinetic studies	Gliomas	215
PCL	Captex Span 80 Tween 80	Melatonin	Nanoprecipitation	166.7 ± 6.3	0.088 ± 0.018	−34.0 ± 5.2		51	Release study Cellular uptake		Pharmacokinetic studies	Glioblastomas	214
PCL	Tween 80	Simvastatin	Emulsification	202.5 ± 18.0	0.12 ± 0.08	−22.2 ± 3.2		99.8 ± 0.7	Release study	Franz diffusion cell Mucoadhesion study		Alzheimer's disease	84
PCL	Methyl methacrylate and 2-(dimethylamino)ethyl methacrylate Medium chain triglyceride	Olanzapine	Self-assembly	254.9 ± 12.1	0.03 ± 0.01	22.2 ± 1.2	0.489 ± 0.021	99.00 ± 0.05	Release study	Residence time evaluation	Pharmacokinetic studies	Schizophrenia	75
PCL	Precirol ATO 5 Geleol monodiglyceride	Resveratrol Methylene blye	Emulsification	326 ± 24	0.344 ± 0.09	−16.45 ± 2.3		96.8 ± 2.8	Release study Mucoadhesion study	Franz diffusion cell		Neuroprotection	180
PCL	Chitosan TPGS	Bromocriptine mesylate	Emulsification	331 ± 3.35	0.17 ± 0.26	21 ± 3.84	5.5 ± 0.5	87.9 ± 0.9	Mucoadhesion study	Franz diffusion cell		Parkinson's disease	165
PCL-PEG	Tat peptide Bombesin	Camptothecin	Emulsification	79.6 ± 17.1	0.4	8.42 ± 1.35		m	Release study Cellular uptake		Pharmacodynamic studies	Gliomas	135
PCL-PEG	Lactoferrin	NAP (NAPVSIPQ, an 8-amino acid neuropeptide fragment derived from the activity-dependent neuroprotective protein)	Emulsification	88.4 ± 7.8	0.22 ± 0.033	−23.56 ± 0.96	0.62 ± 0.013	47.61 ± 2.36	Cellular uptake		Pharmacokinetic and pharmacodynamic studies	Alzheimer's disease	106
PCL-PEG		Bexarotene	Emulsification	120.2 ± 5	0.149 ± 0.03	−19.7 ± 3		66.8 ± 3	Mucopenetration Cellular uptake		Pharmacokinetic studies	Neuroprotection	211
PLA	Poloxamer 188	Methotrexate	Emulsification	351 ± 13.4		−25.1 ± 1.2		58.76 ± 0.54	Release study Mucoadhesion study Franz diffusion cell using a Nylon membrane	Nasal mucosa penetration study (fluorescence)	Pharmacokinetic studies	Glioblastomas	70
PLA	PVA	Thyrotropin-releasing hormone	Emulsification	108 ± 12					Neuroprotection activity		Pharmacodynamic studies	Seizure	69
PLA		Rhodamine	Solvent displacement	152.32 ± 7.87	0.087 ± 0.051	−30.05 ± 0.35			Cellular uptake				68

Table 1 *(Contd.)*

Polymer used	Other molecules	Drug adsorbed	Synthesis method	Size (nm)	PDI	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)	NtB *in vitro* evaluation	NtB *ex vivo* evaluation	NtB *in vivo* evaluation	Application	Ref.
PLA-DSPE-PEG		Zirconium 89	Emulsification	97.1	0.19	−36.0					Pharmacokinetic studies		136
PLA-PEG	Lectin	Coumarin	Emulsification	111 ± 9.8					Release study		Pharmacokinetic studies		110
PLA-PEG	Lectin	Coumarin	Emulsification	85					Release study Nasal ciliotoxicity study		Pharmacokinetic studies		102
PLA-PEG	Lactoferrin	Alpha-asarone	Emulsification	364.5 ± 1.40	0.165 ± 0.017	−21.8 ± 1.01	7.3 ± 0.102	86.34 ± 0.11		Franz diffusion cell	Pharmacokinetic studies Toxicity evaluation	Neuroprotection	124
PLGA	Poloxamer 407	Lamotrigine	Nanoprecipitation	184.6	0.082	−18.8	10.21 ± 0.89	84.87 ± 1.2	Permeation study Cytokine analysis		Pharmacokinetic studies	Neuropathic pain	212
PLGA	Poloxamer 407	Diazepam	Nanoprecipitation	190 ± 0.5	0.31 ± 0.04		11.5	82 ± 0.5		Franz diffusion cell	Pharmacokinetic studies	Epilepsy	174
PLGA	Poloxamer 188 Chitosan	Insulin	Emulsification	174.6 ± 10.7	0.179 ± 0.01	58.4 ± 0.7	2.6	78	Release study Mucoadhesion study Diffusion study across 3D cell culture			Alzheimer's disease	238
PLGA	Tween 80 Chitosan	Rhodamine	Solvent displacement	213.3	0.248	69.4 ± 7.95		10	Release study		Pharmacokinetic studies		143
PLGA	Lecithin DOTAP DSPE-PEG	*trans*-Farnesylthiosalicylic acid	Emulsification	164.3 ± 10.3	0.192 ± 0.06	−12.0 ± 1.3	3.5 ± 0.1	97.7 ± 2	Release study		Pharmacodynamic studies	Glioblastomas	95
PLGA	Poloxamer 407	Olanzapine	Nanoprecipitation	91.2 ± 5.2	0.120 ± 0.018	−23.7 ± 2.1	8.613 ± 0.288	68.91 ± 2.31	Release study	Franz diffusion cell	Pharmacokinetic studies	Schizophrenia	137
PLGA	Lactoferrin Chitosan PVA	Huperzine A	Emulsification	153.2 ± 13.7	0.229 ± 0.078	35.6 ± 5.2		73.8 ± 5.7	Release study Cellular uptake		Pharmacokinetic studies	Alzheimer's disease	239
PLGA	Chitosan	Carmustine	Emulsification	232.5 ± 4.5	0.317 ± 0.170	30.8 ± 6.22	11.7	58.76	Release study	Franz diffusion cell	Pharmacokinetic studies	Glioblastomas	61
PLGA	Tween 80 Glucose	Oxcarbazepine	Solvent displacement	256.16 ± 2.94	0.144 ± 0.024	−15.12 ± 0.36		85.1 ± 2.1			Pharmacokinetic and pharmacodynamic studies	Epilepsy	194
PLGA	Poloxamer 188	Tarenflurbil	Emulsification	133.13 ± 7.82	0.21 ± 0.02	−30.25 ± 2.11		64.11 ± 2.21	Release study		Pharmacodynamic studies	Alzheimer's disease	144
PLGA	Chitosan PVA	Ropinirole hydrochloride	Nanoprecipitation	468 ± 40	0.29	54.4 ± 2.6	5.7 ± 2.5		Release study Mucoadhesion study	Franz diffusion cell		Parkinson's disease	92
PLGA	TPGS	Ropinirole hydrochloride	Nanoprecipitation	279.4 ± 1.8	0.329 ± 0.09	−29.4 ± 2.6	9.2 ± 1.65	72.3 ± 6.1	Release study	Franz diffusion cell Histopathology		Parkinson's disease	173
PLGA		Midazolam	Nanoprecipitation	164 ± 4.5	0.099 ± 0.02	−16.6 ± 2.5			Release study	Franz diffusion cell	Pharmacokinetic studies	Seizure	213
PLGA	Span 80	Frovatriptan succinate	Emulsification	264.4 ± 0.04		−35.17 ± 0.07		65.2 ± 0.06	Release study	Franz diffusion cell Histopathology	Pharmacokinetic studies	Migraine	170
PLGA	PVA	Ephrin type-A receptor 3 tyrosine kinase antibody	Emulsification	145.9 ± 8.7	0.121 ± 0.035	23.08 ± 2.5	3.02 ± 0.68		Release study		Pharmacokinetic and pharmacodynamic studies	Glioblastomas	64
	Chitosan	Temozolomide							Cellular uptake				

Table 1 *(Contd.)*

Polymer used	Other molecules	Drug adsorbed	Synthesis method	Size (nm)	PDI	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)	NtB *in vitro* evaluation	NtB *ex vivo* evaluation	NtB *in vivo* evaluation	Application	Ref.
PLGA	Transferrin Specific peptide against transferrin receptor	Venlafaxine	Emulsification	218.6 ± 0.5	0.078 ± 0.008	−19.5 ± 0.2	10	48	Release study Diffusion study across 3D cell culture		Pharmacokinetic studies	Depression	216
PLGA	Specific peptide against transferrin receptor Poloxamer 407	Agomelatine	Nanoprecipitation	116.06 ± 3.523	0.057	−22.7 ± 2.8	49.15 ± 0.5	98.3 ± 1.067	Release study	Franz diffusion cell Histopathology	Pharmacodynamic studies	Depression	171
PLGA	Chitosan Poloxamer 407	Cetuximab Alpha-cyano-4-hydroxycinnamic acid	Emulsification	258	0.44	37		88			Treatment efficacy on chicken eggs assay	Glioblastomas	161
PLGA		Baclofen Lamotrigine	Nanoprecipitation	177.7	0.057	−15.8	10.2 ± 1.45	85.29 ± 0.81	Permeation		Pharmacokinetic and pharmacodynamic studies	Neuropathic pain	35
PLGA	Tween 80	Tumour necrosis factor (TNF)-related apoptosis inducing ligand	Solvent displacement	543.2 ± 15.78	0.304 ± 0.05	−19.1 ± 0.49		99.81 ± 0.03			Pharmacokinetic studies	Alzheimer's disease	240
PLGA	Chitosan PVA	Duloxetine HCl	Emulsification	122.11 ± 16.22	0.222 ± 0.05	29.8		66.95 ± 1.48	Release study	Franz diffusion cell	Pharmacokinetic studies	Depression	182
PLGA	Transferrin Poloxamer 407	Clonidine	Emulsification	199.5 ± 1.36	0.291	−17.4 ± 6.29	7.8 ± 0.48	86.2 ± 2.12	Permeation across a membrane Cellular uptake	Histopathology	Pharmacokinetic and pharmacodynamic studies	Attention deficit hyperactivity disorder (ADHA)	121
PLGA Poloxamer 407	Poloxamer 407	Lorazepam	Nanoprecipitation	168.2	0.08	−18.4	8.7	90.1	Release study	Franz diffusion cell	Pharmacokinetic studies	Epilepsy	175
PLGA-PEG	*N*-Hydroxy succinimide Borneol	Tanshinone IIA	Emulsification	160		−36	3.6	70	Release study Cellular uptake		Pharmacodynamic studies	Cerebral ischemia/ reperfusion injury	130
PLGA-PEG	Tween 80	Rhodamine B	Nanoprecipitation	190.2 ± 4.1	0.198 ± 0.024	−21.7 ± 0.3		80.19 ± 8.234	Release study Cellular uptake				160
PLGA-PEG	RVG29	miR-124	Emulsification	204	0.4			28.2 ± 8.3			Pharmacokinetic and pharmacodynamic studies	Ischemic brain injury	220
PLGA-PEG	RVG29 Poloxamer 407	Baicalin	Emulsification	89	0.12	−6		64	Release study		Pharmacokinetic and pharmacodynamic studies	Cerebral ischemia/ reperfusion injury	127
PLGA-PEG	Lactoferrin	Rotigotine	Nanoprecipitation	122 ± 19.3	0.194 ± 0.023	−21.28 ± 2.15		92.57 ± 9.41	Cellular uptake		Pharmacokinetic studies	Parkinson's disease	122
PLGA-PEG	Lectin	Coumarin	Emulsification	137	0.14	−30	0.79 ± 0.03	65.87 ± 2.85	Release study Cellular uptake		Pharmacokinetic studies		109
PLGA-PEG	Lecithin Tween 80	Edaravone	Nanoprecipitation	90.2 ± 2.2	0.214 ± 0.011	−11.9 ± 1.14	3.02 ± 0.32	20.58 ± 2.18	Release study Activity evaluation		Pharmacokinetic studies	Amyotrophic lateral sclerosis	87
Pluronic L121 Pluronic P123		Olanzapine	Thin-film hydration	58.55 ± 2.47	0.27 ± 0.03		1.84 ± 0.06	75.03 ± 2.35	Release study	Franz diffusion cell	Pharmacokinetic studies	Schizophrenia	241
Polystyrene	Chitosan			163.0	0.08	30.1 ± 2.0					Pharmacokinetic studies		199

to improve properties enhancing NtBD, such as adherence to the olfactory epithelium, avoidance of mucociliary clearance, and protection against enzymatic degradation. However, lipid-based NPs have a limited drug-loading capacity and need to be further studied.[16]

Finally, polymeric NPs can be composed of various polymers, with different natures and properties. These polymers can be chitosan or polylactic acid for example. Their variable composition allows a fine adjustability in their design, as size, charge, and surface chemistry can be controlled.[17] These polymeric NPs are extensively used for various applications due to their simple elaboration, strong biocompatibility, and biomimicking characteristics.[18]

In this review, we will focus on polymeric NPs that are specifically used for NtBD, with a particular emphasis on their use in treating central nervous system disorders, such as Alzheimer's disease[19] or Parkinson's disease.[20] We will discuss the composition and physicochemical characteristics of the NPs used, especially polymers and other molecules added to functionalize the polymeric NPs. Then, *in vitro*, *ex vivo* and *in vivo* NtBD evaluation will be presented to explore the different ways to characterise NPs for the NtBD.

Data found in the articles selected are summarised in Table 1 that details polymers, as well as the drug in the NPs. We also detailed the synthesis method, physicochemical characteristics, evaluation methods used that are specific to the NtBD and finally the applications of these polymeric NPs used for the NtBD.

2. Nose-to-brain delivery

2.1. Nasal cavity anatomy

The human nasal cavity is the first element of the respiratory system. Its main roles are respiration and olfaction. The nasal cavity is lined with a mucous membrane rich in blood vessels, facilitating rapid warming and humidification of inhaled air. The nasal cavity is composed of three main areas: the vestibular area, the olfactory area, and the respiratory area (Fig. 1).[21]

First, the vestibular region is the closest to the outside. It is composed of a squamous epithelium that protects from irritation due to a large production of mucus and the presence of hairs (Fig. 1A).[21]

Then, the olfactory region is about 3% of the human nasal cavity surface (Fig. 1B). The olfactory mucosa is a pseudostratified columnar epithelium composed of olfactory sensory neurons (OSN), sustentacular cells, microvillar cells, and basal cells.[22] OSN are bipolar unmyelinated neurons whose cell bodies are located in the epithelium of the olfactory mucosa. They project cilia in the nasal cavity, protected by the mucus layer, that express olfactory receptors sensitive to odorant molecules. The axons of OSN cross the cribriform plate to reach the olfactory bulb where they do synapses with other neurons in the brain.[23] Sustentacular cells are located next to OSN and they regulate and maintain homeostasis around neurons.[22] Microvillar cells are supposedly chemoreceptors, but their precise role has not been fully described yet. Basal cells are stem cells located close to the basal lamina, that can regenerate OSN.[22] Bowman's glands are

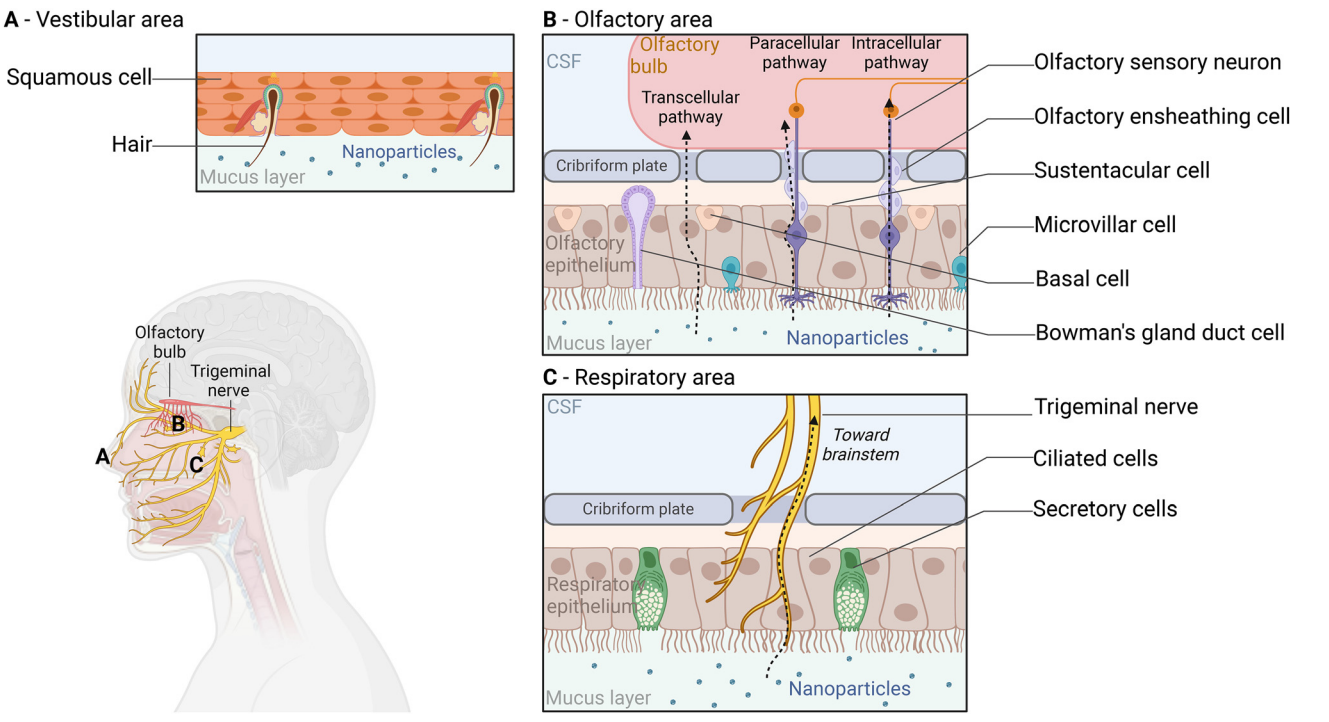

Fig. 1 The different epithelia of the nasal cavity and nose-to-brain pathways. The most anterior area is the vestibular area (A). The olfactory region (B) is the main region where NtBD occur. The major part of the nasal cavity is respiratory area (C) where NtBD can occur through the trigeminal pathway (CSF: cerebrospinal fluid). Created with BioRender.com.

also found in the olfactory epithelium and are responsible for the mucus secretion.[22] The olfactory region is also composed of olfactory ensheathing cells that contribute to the electrophysiology maintenance of mature OSN but that also participate in their regeneration.

Finally, the respiratory region covers about 85% of the nasal cavity (Fig. 1C). This is a pseudostratified columnar epithelium composed of a large variety of cells including ciliated cells and secretory cells such as goblet cells or seromucous cells. The ciliated cells have motile cilia that move the mucus through the entire mucosa. The secretory cells produce a major part of the nasal secretions, both mucus and enzymes, that act as a filter against pathogens and allergens that can be found in the inhaled air. The respiratory mucosa is innervated by the trigeminal nerve, which also has also terminations in the vestibular and olfactory epithelium.[23,24]

These different regions, through the diversity of their cellular composition, possess specific characteristics contributing to particular physiological functions. Their proximity with different neuronal pathways makes the nasal cavity an interesting entry point to reach the brain. Several ways can be taken by drugs to reach the brain from the nasal cavity.

2.2. Nose to-brain pathways

As mentioned earlier, there are different connections between the nasal cavity and the brain. Nasal administration of drugs can occur *via* different pathways that can be either direct, *via* OSN or the trigeminal nerve, or indirect (Fig. 1).

The olfactory pathway is based on the presence of 6 million OSN in the human olfactory mucosa.[25] Molecules that reach the olfactory epithelium can be either internalized *via* endocytosis or passive diffusion by the sustentacular cells (transcellular pathway), cross the tight junctions (TJs) between cells (paracellular pathway), or be taken up into the neuronal cells by endocytosis or pinocytosis and transported alongside the axon to the synapse in the olfactory bulb (intracellular pathway) (Fig. 1).[26] In all cases, drugs or NPs can enter the olfactory bulb and the CSF to be distributed in the brain.[27]

The trigeminal nerve pathway can take place in the olfactory epithelium as well as in the respiratory epithelium. Molecules must first diffuse through the mucosa, and reach the brainstem, either *via* intracellular or extracellular route. It was showed that the trigeminal nerve has direct connection with the nasal mucosa, and it allows NPs delivery to the brainstem.[28] For example, it has been proven that fluorescence was visible in the trigeminal nerve 1 hour post-INA of fluorescent microspheres, and in the trigeminal ganglion (Gasser) more than 7 days after administration.[24]

Once NPs reach the olfactory bulb *via* the olfactory pathway or the brainstem *via* the trigeminal pathway, diffusion to the rest of the brain is necessary. This transport seems to occur due to extracellular flow alongside both nerve bundles but the complete mechanism is not fully understood.[7]

If NPs are not able to penetrate cells and remain in the extracellular environment, they can be absorbed by blood vessels or lymphatic vessels.[7] Indeed, as the nasal mucosa is well vascularized, NPs administered in the nose can diffuse through the olfactory or the respiratory mucosa and reach the blood by crossing the endothelium. This indirect adsorption mechanism allows NPs to reach the systemic circulation. NPs must then cross the BBB to attain the brain.[29]

2.3. Advantages

NtBD presents numerous advantages, the main one being circumventing the BBB as mentioned earlier. The BBB is one of the most difficult barriers to cross. It is composed of endothelial cells, pericytes, and astrocytes, forming a selective barrier between the brain and the blood. Endothelial cells surround blood vessels and are linked by both tight and adherent cell junctions, limiting molecules crossing. Pericytes are localised around endothelial cells and express both gap junctions and adhesion plaques. Finally, astrocytes are glial cells localized around pericytes and link vascular and neuronal cells. They also express tight junctions. The superposition of these three cell types that express strong junctions in between them and with each other lead to an efficient barrier preventing passage of large molecules.

The transport of some molecules such as ions, neurotransmitters, and nutrients is possible but molecules weighing more than 450 Da are not able to cross passively the BBB.[5] The TJs expressed by the endothelial cells are specific and selective, active transport is therefore necessary for molecules to cross the BBB.[30,31] This selectiveness impacts the brain bioavailability of drug administered *via* a route of administration where the drug reaches the systemic circulation, such as oral or IV route. By also preventing its degradation by digestive enzymes or avoiding the first-hepatic passage, NtBD allows the enhancement of the drug's bioavailability and the local drug concentration. This implies a higher amount of the drug delivered to the brain, compared to other ways of administration.[32]

Then, NtBD allows fast adsorption of the drug mainly due to a large surface area thanks to cilia, and a quite thin epithelium.[33] It also reduces drug's blood distribution, therefore minimizing the risk of systemic side effects.[34] For example, when Nigam *et al.* designed NPs for NtBD, they found out that the drug was more abundant in the brain after INA compared to IV administration, even if the dose administered was the same.[35]

Moreover, INA offers a non-invasive, painless, safe, and easy administration technique, that increases patient compliance and facilitates repeated administrations.[36] In case of emergency, this route can also be used on unconscious patients. It has been reported that the nasal route is preferred by both patients and caregivers over other administration routes.[37]

The intranasal route, *via* the different pathways, offers numerous advantages, using the unique nasal epithelium for rapid absorption and direct access to the central nervous system, bypassing the BBB and reducing systemic side effects.

2.4. Limitations

Despite many advantages, some drawbacks must be taken into account when developing therapeutics for NtBD. One of the

main barriers between the nasal cavity and the mucosa is the mucus. Other biological limitations such as mucociliary clearance, enzymes, or more technical limitations should be considered when designing NPs for NtBD.

2.4.1. Mucus. Mucus is essential for the airways' health and functionality. It is mainly composed of glycoproteins called mucins, but also salts, lipids, DNA, cells and cellular debris.[38] There are 13 different mucins, secreted majorly by goblet and seromucous cells, but only seven are predominant: four membrane-associated (Muc1, Muc4, Muc16, and Muc20), one secreted (Muc7) and two gel-forming (Muc5AC and Muc5B).[39] The cell surface-associated mucins are directly related to the epithelium, creating a barrier and coordinating the cilia movements. The secreted mucin's role is to retain water. The gel-forming mucins trap particles that must be cleared and evacuated.[40] This gel network has pores of 150 ± 50 nm of diameter that influences mucus permeability. Indeed, mucus is a semi-permeable barrier capable of filtering molecules due to their size or by interacting with them.[38] Mucus and mucins have, therefore, a huge role in immunity by being a barrier but also interacting with the dendritic cells and mediating inflammatory cascade pathways.[40] The olfactory epithelium is different from the respiratory epithelium due to mucins' differential expression, as the olfactory epithelium expresses Muc1, Muc5AC and Muc5B differently from the respiratory epithelium. For example, Muc1 is consistently expressed at the surface of the olfactory epithelium whereas it is more patchy in the respiratory mucosa.[39]

Mucus in the nasal cavity is 10 to 15 μm thick and is composed of two layers, one more liquid close to the epithelium and one in contact with the lumen that is thicker.[41] Ionic strength and pH of around 6 also impact filtration by modifying the tightness and viscoelasticity of the gel network. Changes in pH can modify interactions between mucins and increase viscoelasticity. A more basic pH tends to reduce the viscosity and increase the nasal mucosa crossing.[38]

Designing NPs should consider all these characteristics: the charges due to mucins, the filtering capacities, the pH and the ionic strength.

2.4.2. Mucociliary clearance. Mucociliary clearance is also a factor that influences nasal adsorption. This mechanism is crucial for the defence against any foreign substance. The main actors of mucociliary clearance are the cilia at the surface of the epithelial cells. The cilia beat in a coordinated and unidirectional way and can eliminate non-specifically every molecule.[42] The mucociliary clearance is of 12 to 15 min.[43] Indeed, some molecules adhering strongly to the negatively charged mucus could be carried out to the nasopharynx and eliminated by deglutition which may be challenging for the nasal adsorption of therapeutic drugs.[44]

NPs for the NtBD should be sufficiently penetrating in the epithelium and the brain to avoid being eliminated by mucociliary movements. Different strategies of mucopenetration can be used, in combination with NPs, such as administration devices, or mucoadhesive surface modifications.[45]

2.4.3. Enzymes. In the respiratory region of the nasal cavity, goblet and seromucous cells secrete enzymes that protect the airways from the contamination by volatile pathogens. Most of the enzymes of the nasal cavity, such as proteases and peptidases, are present in higher concentrations at the olfactory epithelium.[46] Besides metabolizing drugs, enzymes can modify their solubility profile, alter their activity, or reduce their permeation properties which can influence their passage from the nasal cavity to the brain.[43] Among these enzymes, P-glycoprotein, an efflux pump, cytochrome P450 and other proteolytic enzymes can form a "pseudo-first pass effect" and can reduce the amount of drug delivered to the brain.[46]

Thanks to their intrinsic properties, NPs offer a significant advantage in nasal drug delivery by protecting therapeutic agents from enzymatic degradation in the nasal cavity, ensuring greater drug stability and controlled release for efficient transport to the brain.

2.4.4. Administration tools. Even if INA seems to be very promising for NtBD, devices need to be designed to deliver the drug in the correct nasal area. The design of such nasal spray remains a challenge as the droplets size, the spray mode, and the single actuation content can impact the deposition location. Surface area can also affect the adsorption of the drugs or the NPs.[47] For example, it has been proven that droplets with a size of around 20 μm will be more concentrated in the anterior region of the nasal cavity. On the contrary, when the droplet size is around 10 μm, they tend to penetrate deeper into the airways, reaching the lower respiratory tract.[9] But even if these nasal spray devices can deliver drugs to different parts of the nasal cavity, a more focused administration to the olfactory mucosa is necessary to ensure a strong bioavailability of the drug in the brain.[48]

To conclude, despite numerous advantages, NtBD faces some drawbacks and one way of overcoming these challenges is the use of NPs, more specifically polymerics NPs, which can adapt to the presence of mucus and mucociliary clearance, protect the drug from enzymatic degradation, and enhance retention in the nasal cavity, thereby improving drug delivery efficiency.

3. Polymeric nanoparticles in the nose-to-brain delivery

As mentioned earlier, lipid-based NPs, inorganic NPs and polymerics NPs have all been explored for NtBD. This review focuses on the latter, due to their unique advantages. Composed of one or more polymers, these NPs stand out for their tuneable properties, straightforward design, and excellent biocompatibility. The wide variety of available polymers enables the creation of diverse designs with tailored chemical and biological functionalities.[15] Notably, polymeric NPs allow precise control over surface charge and particle size, critical parameters for effective NtBD. Their surface can be modified or functionalized to enhance desirable features, such as

mucoadhesiveness or cell-penetration capabilities, improving their interaction with the nasal environment. Furthermore, polymeric NPs provide a versatile platform to address the limitations of the nasal cavity, offering potential solutions to challenges like enzymatic degradation and mucociliary clearance.[44]

Today, no strong correlation exists between the polymeric composition of the NPs and the NtB pathways as described earlier. For example, it has been demonstrated than poly (lactic)co-glycolic acid NPs were able to reach the brain by the transcellular route, in between cells by disrupting TJs.[49] It was also showed that the same NPs were located in different areas of the nasal cavity according to their size, suggesting the use of different pathways.[50] Despite the lack of consensus on the different pathways used by polymeric NPs, some authors have described in more depth the mechanisms of NtBD, that will detailed along the next sections.

3.1. Polymers as nanoparticle building blocks

Different polymers can be used as nanoparticle building blocks as mentioned in Table 1 (Fig. 2 and 3).

3.1.1. Chitosan. Chitosan is a linear polysaccharide naturally derived from chitin deacetylation. It is obtained from crustacean or insect shells, fungus, yeast, or algae.[51] Chitosan is becoming more and more used for medical and pharmaceutical applications, it is found in approved products for antibacterial effect or wound healing.[52]

Chitosan is one of the most used cationic polymers in NtBD. The most commonly used chitosan has a low molecular weight, allowing the design of smaller NPs as compared to higher molecular weight chitosan.[53] This use of chitosan for NtBD NPs is mainly due to its advantageous properties. It is considered nontoxic, biocompatible, biodegradable, and has antibacterial properties.[54] Chitosan is also very interesting for NtBD for its mucoadhesive properties. At an acidic pH, its positive charges interact with the negative charges of mucins and with the cell junctions, allowing its retention in the nasal cavity.[55] When the epithelium is in contact with chitosan, proteins from TJs are redistributed, allowing the opening of these TJs and the passage of molecules. This protein redistribution is reversible, chitosan does not have a long-term impact on the epithelium.[54] Chitosan can be modified by adding different molecules due to *N*-acetylated and deacetylated glucosamine functions. For example, chitosan can be reshaped into thiolated chitosan to enhance both its mucoadhesiveness and its permeation. The latter is explained by the covalent linkage between thiolated chitosan and the mucus layer.[56] Conversely, one main drawback of using chitosan is its poor solubility in water at a neutral or basic pH. In the context of NtBD, chitosan solubility can be improved in the acidic nasal mucus by chemical modifications.[57]

Bhattamisra *et al.* designed 75 nm chitosan NPs encapsulating rotigotine for the treatment of Parkinson's disease. When comparing INA of the free drug and the drug loaded in these positively charged chitosan NPs, the latter had a stronger pharmacological effect than the drug alone on animal model.[58] Similarly, Shukr *et al.* loaded agomelatine in 190 nm positive chitosan NPs for its anti-depressive activity and found out that the drug concentration was increased in the brain after INA of NPs.[59] Uppuluri *et al.* designed 148 nm positive chitosan NPs loading piribedil in a methylcellulose gel for INA and after *in vivo* administration, both NPs and NPs in the gel showed a stronger brain availability compared to the drug alone.[60] These three studies showed that chitosan NPs were an efficient alternative to using the free drug for NtBD. Moreover, chitosan can also be used as a coating onto preformed NPs.[61]

3.1.2. Poly(lactic)co-glycolic acid. Synthetic polymers such as poly(lactic)co-glycolic acid (PLGA) have been extensively studied since they have higher reproducibility and purity than natural polymers. PLGA is commercially available and is approved by both the Food and Drug Administration (FDA) and the European Medicine Agency.[62] PLGA is a biocompatible and biodegradable polymer as its monomers are endogenous and part of the citric acid cycle. Due to its structure, the molecular weight and the copolymer ratio can vary to tune the NPs' size, capacity of entrapment, release ability, and biocompatibility.[63]

Indeed, PLGA NPs are developed for NtBD of treatments for several pathologies. Chu *et al.* used 146 nm PLGA NPs to show the efficacy of their treatment on the survival of rats with gliomas. They demonstrated that their positive formulation was localised in the brain after INA and that it improved rat survival.[64] PLGA NPs can have a high encapsulation capacity as Nigam *et al.* designed PLGA NPs, that were 178 nm and positively charged, encapsulating two drugs, baclofen and lamotrigine, to target neuropathic pain. They showed that, not only high drug concentration in the brain was achieved, but also that these PLGA NPs had a pharmacological effect *in vivo*

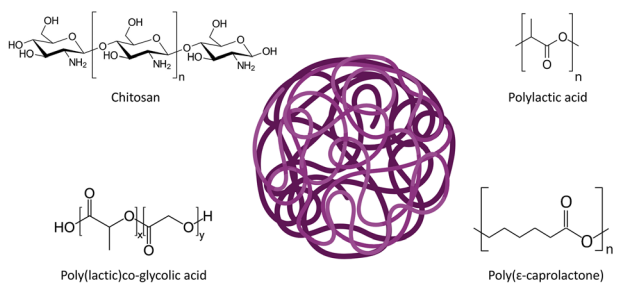

Fig. 2 Different polymers can be the core of the nanoparticle for NtBD. Created partially with BioRender.com.

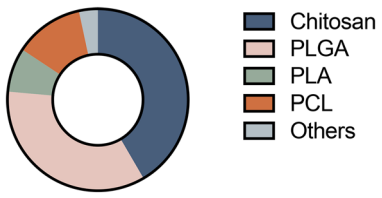

Fig. 3 Polymers used as building blocks for NPs used for NtBD.

after INA.[35] Finally, these NPs can be used to adsorb larger molecules such as antibodies. Musumeci *et al.* described the adsorption an antibody at the surface of PLGA NPs targeting amyloid beta protein physiopathology in AD. After INA of functionalized negative 543 nm NPs, antibodies were found in the mice brain.[65] PLGA NPs are very promising for the NtBD as they are quite versatile in terms of drugs absorbed or encapsulated that could be a simple molecule, two drugs, or even an antibody.

3.1.3. Polylactic acid. Polylactic acid (PLA) is a polymer of great interest in the medical field as it is both biocompatible and biodegradable. Due to its adequate properties, PLA has been approved in the US by the FDA for several applications such as implants and prostheses. PLA is also non-toxic and has been used for the preparation of targeted drug delivery systems. PLA is hydrophobic and is therefore a good candidate for encapsulating hydrophobic drugs, protecting them from degradation.[66] Physicochemical properties can be controlled to obtain the desirable NP characteristics. Plus, in PLA NPs, the active agent is often homogenously dispersed resulting in a constant drug release.[67] Musumeci *et al.* prepared PLA NPs, PLGA NPs, and chitosan NPs of respectively 152 nm, 133 nm and 181 nm. PLA and PLGA NPs were negatively charged and chitosan NPs were negatively charged. They compared internalisation in olfactory ensheathing cells and concluded that PLA NPs and chitosan NPs were more suitable for the NtBD, but the chitosan NPs were bigger and less homogenous than PLA NPs.[68]

PLA NPs can also be used to deliver different types of molecules. Veronesi *et al.* used PLA NPs to deliver thyrotropin-releasing hormone as an anti-epileptic, that were 108 nm and no charge was mentioned. After INA, they demonstrated the positive impact of drug-loaded PLA NPs on seizures appearance.[69] Moreover, Jain *et al.* designed methotrexate PLA NPs in a thermosensitive hydrogel to treat glioblastoma. They measured their NPs at 351 nm and −25 mV. They showed that both the free and the loaded drug in PLA NPs could reach the brain similarly 2 h after INA, and that only the loaded drug was still present 24 h after administration. Therefore, they showed the ability to target the brain with PLA NPs but also a small retention effect.[70] These studies demonstrate the potential and the flexibility of using PLA as the polymeric core of NPs in the context of NtBD.

3.1.4. Poly(ε-caprolactone). Poly(ε-caprolactone) (PCL) is a polymer formed by an aliphatic polyester chain. It is biodegradable and can be used for drug delivery systems,[71] and because of its high hydrophobicity, PCL can aggregate into NPs.[72] These PCL NPs are developed for NtB treatments targeting a large variety of diseases. Sawant *et al.* used aripiprazole-loaded PCL NPs to treat schizophrenia, and after INA, they showed enhanced brain targeting efficiency allowing dose reduction and toxicity limitation.[73] Alex *et al.* developed carboplatin-loaded PCL NPs of 312 nm and negatively charged. They showed that NPs were able to bind the nasal mucosa and have better nasal adsorption than the drug alone.[74] Finally, Fonseca *et al.* designed positive PCL NPs of 255 nm to deliver olanzapine to the brain and demonstrated that the drug remains biologically active with an increased efficacy thanks to the encapsulation in PCL NPs.[75]

All of these polymers have been largely studied for NtBD due to their biocompatibility and their biodegradability. Plus, polymeric NPs can penetrate the mucus, enhance the contact time with the nasal mucosa, limit the impact of mucociliary clearance, and protect the drug from enzymatic degradation.[76] Depending on the polymer chosen to compose their core, NPs can be either positively or negatively charged and their size can be modulated. But even if they are relevant for the NtBD on their own, they are often functionalised either by modification of the polymer (chemical grafting of a functional moiety) or by addition of another molecule in the synthesis to change or strengthen their properties to further improve NtBD.

3.2. Formulation and functionalisation of polymeric nanoparticles

NPs polymeric core can be modified and grafted with other molecules to enhance the formulation by increasing both stealth and stability, balancing both mucoadhesion and mucopenetration and enhancing nasal mucosa penetration to improve brain bioavailability. A summary of molecule that were used is presented in Table 1 and in Fig. 4.

3.2.1. Stability and stealth. Different molecules can be added to facilitate the formulation of NPs and their stability. *In vivo*, the surface hydrophobicity of certain polymeric NPs can activate a host immune reaction triggering NPs degradation. One of the main phenomena is the opsonisation, where blood proteins bind to the NPs leading to phagocytosis by macrophages, thus reducing the number of NPs reaching the target, herein, the brain. Indeed, the high vascularisation of the nasal mucosa can lead to partial blood delivery of NPs. Designing NPs capable of avoiding opsonisation and immune response is essential to prevent side effects. One way of creating these stealth NPs is to add a hydrophilic molecule at the surface like surfactants or other molecules.[77]

3.2.1.1. Surfactants. Surfactants are composed of a head and a tail, that can be either hydrophobic or hydrophilic. All surfactants have a hydrophilic part and a hydrophobic one. Due to the charge of their head, surfactants can be dispatched into different categories: positively charged, negatively charged, zwitterionic and non-ionic surfactants. Non-ionic surfactants are less toxic as they are non-ionizable in aqueous solutions, making them the most used in nanotechnology.[78]

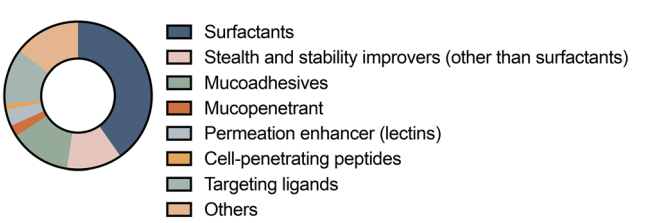

Fig. 4 Functionalisation of polymeric NPs for the NtBD.

The amphiphilic nature of surfactants is ideal to improve the NPs stability and stealth. Their hydrophobic part stays in contact with the core of the NPs when their hydrophilic part remains in the water. Adsorption of a surfactant at the particle surface or addition of the surfactants in the NPs synthesis can also impact stability but also physicochemical characteristics. It has been proven that the modification of the surfactant tail length can result in a smaller or a larger particle, it can also limit the aggregation of the NPs, improving their stability.[79]

Poloxamers are non-ionic surfactants composed of hydrophilic poly(ethylene oxide) and hydrophobic poly(propylene oxide), which proportions can vary. The hydrophobic part is in contact with the NPs whereas the more hydrophilic part is more exposed at the NPs surface.[80] Due to their two-blocks properties, they can create a stealth effect due to the inhibition of opsonisation by building a hydrophilic coat onto the NPs.[78] It was also proven that the adsorption of poloxamer 188 at the surface of NPs increased the steric stability due to the repulsive hydration forces at the surface of the NPs.[81] It was also shown that NPs coated with poloxamer 407 disperse more rapidly into the mucus layer than bare NPs inhibiting the contact between the NPs core and the mucus.[82] NPs coated with poloxamer 188 showed a faster and higher uptake by cells than NPs coated with polysorbate 80.[83]

Similarly to poloxamers, polysorbate 80 (or Tween 80) is a non-ionic surfactant that is often used for stabilizing NPs.[84] Polysorbate 80 has also been proven to target the brain, resulting in increased bioavailability of the encapsulated drug.[85]

D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) can also be used as a surfactant. It is derived from the vitamin E and polyethylene glycol (PEG). It can be used as an emulsifier, a stabilizer, a penetration enhancer, and in micelle protection. As it is completely miscible with both water and oil, it is widely used for its stabilization properties. To be noted that TPGS can also inhibit the P-glycoprotein, found at the surface of the nasal cavity, preventing efflux from the cell.[86]

Lecithin is a natural and biocompatible surfactant that has emulsifying properties.[59,87] It was used as a stabilizer and improved the adsorption of proteins at the surface of lipophilic NPs by creating a phospholipid monolayer at the NPs surface that is known to improve NPs biocompatibility.[88] Lecithin was added in the synthesis of chitosan NPs and it was showed that NPs were more concentrated in the brain than the drug suspension after INA.[59]

Hybrid NPs are a combination of a polymeric NPs core and a lipid layer composed of a biocompatible lipid and a more stealth lipid. As a result, these hybrid NPs have a great structural integrity, biocompatibility and bioavailability provided by the lipid layer. Their main advantage is the increased stability and circulation time due to the biomimetics of the lipids. Additionally, different drugs can be encapsulated, both hydrophilic and hydrophobic.[89]

Thus, the use of surfactants on the surface of polymeric NPs considerably improves their stability and functionality, facilitating better interaction with biological environments and preventing aggregation in the nasal cavity environment.

3.2.1.2. Others molecules. Other molecules can enhance NPs stealth. PEG is one of the most widely used molecules to functionalize NPs. It can be added as a coating of the NPs surface or covalently bounded to the polymer composing the NPs. PEG has numerous advantageous characteristics, it is biocompatible, biodegradable and it can protect nanoformulations from biological and chemical degradation. PEG can have different molecular weight from 2 to 20 kDa, the larger the PEG, the less aggregation. It was showed that covering PLA NPs with PEG larger than 5 kDa decreased proteins adsorption compared to non-PEGylated NPs. PEGylation improves the cellular uptake of the NPs and also shows increased stability and transcytosis resulting in improved adsorption across the nasal mucosa. Besides, the addition of the PEG on polymeric NPs confers mucoadhesive properties to the drug delivery system.[90] Despite being a very interesting ingredient for NtBD due to its properties, more and more hypersensitivity reactions seem to be associated with PEG.[91]

Polyvinyl alcohol (PVA) is often used with chitosan NPs. It is a co-block polymer that has both hydrophilic and hydrophobic parts. This molecule shows steric repulsion when adsorbed at the surface of NPs, therefore improving their stability.[92]

Human serum albumin can also be used to coat NPs and improve their stability. Albumin has low immunogenicity and is biocompatible. Besides being able to target tumour cells,[93] albumin-coated NPs show greater stability and thus, higher bioavailability as the corona prevents opsonisation and elimination of the NPs.[94]

Different molecules can be added to increase both stability and stealth of NPs. These molecules can be combined to further ameliorate NPs properties for a more efficient NtBD delivery. For example, Sekerdag *et al.* formulated NPs using PLGA and an anticancer salicylic acid derivative drug. Then, they added lecithin, a natural surfactant, plus a mono cationic lipid and an amphiphilic lipid linked to PEG. Only four hours after INA of their hybrid NPs, the drug was significantly more present in the olfactory bulb compared to the drug alone. Plus, there were less NPs in the liver and the spleen after INA than IV administration, suggesting that both the addition of a lipid layer onto the NPs and INA not only did increase the drug concentration in the brain but also limit the risk of potential side effects in the spleen and the liver.[95]

3.2.2. Mucoadhesiveness. To limit the impact of mucus, as well as of mucociliary clearance, mucoadhesiveness can be improved using different molecules at the surface of the polymeric NPs. Mucoadhesion can be described as the interaction with mucins whereas bioadhesion is the interaction with biological membranes.[96] As the mucus in the nasal cavity is negatively charged, most mucoadhesive molecules are positively charged to create electrostatic interactions. By these interactions, the mucoadhesive molecules allow a longer residence time of the NPs in the nasal cavity, thus increasing their uptake across the nasal mucosa.[96]

Chitosan is one of the main mucoadhesive molecule used for NtBD since it has numerous advantages that makes it suitable for INA. It can be used as a polymer composing the NPs

or as a coating of an already formed polymeric NPs, as mentioned earlier (see section 3.1.1.). Chitosan has been widely used as a mucoadhesive agent but also a penetration enhancer to obtain a longer retention time at the nasal mucosa. By modifying the charge of the NPs into a positive one, chitosan allows electrostatic interaction between the positive NPs and the negative nasal mucosa, and mucoadhesion.[92]

Other mucoadhesive molecules could have been studied such as pectin that is a polysaccharide that can be used to coat NPs to further enhance mucoadhesion.[97] Alginate could also be a great candidate for mucoadhesion as it has already been used to form NPs with lipids.[98] Other molecules could have also been used to improve adhesion to the nasal mucosa such as Carbopol, carboxymethyl cellulose or polyacrylates.[99]

Modifications of some preexisting polymers can enhance their mucoadhesion properties: maleimide functionalisation, thiolation, acrylate or methacrylate addition can be used.[100] For example, Singh *et al.* used thiolated chitosan NPs to encapsulate an antidepressant drug and showed that after INA, that the treatment was very effective on animals.[101] Similarly, Gao *et al.* used modified maleimide-PEG-PLA NPs for NtBD. They showed that their NPs were found in the brain after INA.[102]

3.2.3. Mucopenetration. As mucoadhesion is of great interest to prolong the residency time of NPs in the nasal cavity, too strong of a mucoadhesion can also slow down or even stop the NPs from reaching the brain. Different molecules can be added to balance mucoadhesiveness by limiting any interactions between NPs and mucus. Indeed, as too strong mucoadhesion can cause faster clearance of the NPs, a balance must be found.[23]

One of the most prominent molecules used is PEG. The goal is to used either negatively or densely charged molecules, to have the most neutral charge and limit the mucus entrapment.[103] For INA, PEG can be used as a mucus penetration enhancer, as it hydrophilic characteristics prevent interactions with the mucus therefore enhancing the particle diffusion and the mucus penetration.[104] PEG-coated NPs are more protected against chemical and biological degradation due to its inertia in biological media, with low protein adsorption.[105] However, the PEG steric hindrance can prevent it from interacting with the cell membrane. Therefore, it can be useful to add molecules that can allow a better cellular uptake.[106]

To summarise, designing NtBD systems is finding the right balance between mucoadhesion and mucopenetration. Even if these two properties seem contradictory, it has been found that of combination of both mucoadhesion and mucopenetration may be relevant for NtBD.[104]

3.2.4. Permeation enhancer. After the mucus layer, NPs still have to cross the olfactory mucosa to reach the brain, that's why the use of permeation enhancer at the surface of the NPs is important. They can improve mucosal crossing, either by improving cellular uptake or by allowing a higher permeation through TJs. Cellular uptake can be improved by disrupting membranes or using molecules that can bind to receptors and trigger endocytosis.[107] On the other hand, cationic polymers such as chitosan are known to interact with TJs and loosen them. Anionic polymers can also have permeation enhancement properties by decreasing transepithelial electrical resistance (TEER).[107] In this section, we will focus on improving cellular uptake, as one of the most interesting pathways for the NtBD seem to be entering the olfactory nerve. This pathway is the most direct one as other pathways imply a liberation in the lamina propria and a risk of NP loss in the extracellular space.[108]

3.2.4.1. Lectins. Lectins are glycoproteins with a domain able to bind to saccharides and carbohydrates at the cell membrane. This linkage with carbohydrates can induce internalization in the cell. To improve nasal adsorption of NPs, several lectins, that target receptors in the olfactory mucosa, can be used: *Solanum tuberosum* lectin, wheat-germ agglutinin (WGA), and *Ulex europeus* agglutinin I (UEA-I). These molecules can be conjugated with NPs to act as binding molecules. *Solanum tuberosum* lectin can specifically bind to *N*-acetylglucosamine that is abundant in the nasal cavity, especially in the olfactory region. It was adsorbed at the surface of PEG-PLGA NPs allowing them to be better distributed in the brain compared to NPs without lectins.[109] WGA can also specifically bind to *N*-acetyl-D-glucosamine and sialic acid that are observed in the nasal cavity. Gao *et al.* adsorbed WGA onto PEG-PLA NPs and they showed that the functionalisation of NPs increased the adsorption in the brain.[102] The same authors also used UEA-I as a NPs surface modification. It can specifically bind to L-fructose that is largely present at the surface of the olfactory mucosa.[110] These three studies show that adding lectins onto NPs can improve NtBD and brain bioavailability.

3.2.4.2. Cell penetrating peptides. Cell penetrating peptides (CPPs) are small proteins able to cross cell membranes and to translocate their cargo directly into the cell. They can help molecules cross the nasal mucosa without having a specific receptor. This makes them very promising for the functionalisation of polymeric NPs for NtBD. However, the major drawback of using CPPs is their instability *in vivo*.[111] The second drawback is the inability to deliver the load in the cytosol. These two issues can be overcome by chemically modifying the CPP, by *N*-acetylation or *C*-amidation. Finally, the third disadvantage of using CPPs is the lack of cell selectivity. Some new CPPs have successfully been used to target specific organs mainly by reacting with some environmental characteristics (tumour microenvironment for example).[112] Different CPPs have been used for NtBD, such as the transactivator of transcription (TAT)-derived peptide, protamine or penetratin.

One of the most used CPP is the transactivator of transcription (TAT), a protein from the human immunodeficiency virus type I. Its basic core segment of 11 amino acids, named TAT-peptide, can promote the cellular uptake of peptides, proteins, and drugs. Its main cell-penetration mechanism is endocytosis.[113] Kanazawa *et al.* used NPs composed of PCL and PEG grafted or not with TAT-peptide. After INA of these NPs, they observed that NPs were more abundant in the brain 4 h after administration when grafted with TAT-peptide suggesting the relevance of this peptide for NtBD.[114]

Protamine, another CPP, is a drug often used in medicine as an anticoagulant but it can lead to some adverse respiratory effects, such as bronchospasms or pulmonary oedema. To overcome these adverse reactions, low molecular weight (LMW) protamine was created based on the amino acidic sequence. This new peptide is composed of 15 amino acids, which are mainly arginine residues. The adverse side effects were reduced and the peptide showed a structural similarity with the TAT peptide suggesting its activity as a CPP.[115] This new peptide has been shown to be non-antigenic and non-mutagenic. INA of NPs of PEG-PLGA coupled or not with this LMW protamine were performed in rats and the presence of protamine increased the presence of NPs in the brain.[116] These CPP can be of great interest in the context of NtBD.

Penetratin is an interesting amphipathic CPP of 16 amino acids derived from the Antennapedia protein homeodomain and that is already used on NPs and more specifically on liposomes. It was demonstrated that L-penetratin helps the delivery of peptide drugs from the nose to the olfactory bulb using the olfactory mucosa pathway.[117] It has also been proven that INA of L- or D-penetratin with insulin ensure an efficient NtBD, the latter reaching deep region of the brain.[118]

3.2.4.3. Targeting ligands. One of the main advantages of using NPs is to perform a targeted drug delivery, maximizing therapeutic effects and minimizing side effects.[119] This can be further enhanced by functionalizing NPs with targeting ligands, which enable specific interaction with receptors expressed on the cells of interest. In the context of NtBD, these ligands can facilitate the bypassing of physiological barriers and direct the drug payload more effectively to neuronal tissues, improving precision and therapeutic outcomes.

These targeting ligands can be transferrin receptors ligands. Transferrins are a family of glycoproteins divided into two main types: serum transferrins, expressed in blood and lactoferrins, found in milk, tear, saliva and other secretions. Their role is linked to their ability to bind iron.

Serum transferrins act as iron transporters in the systemic circulation and lactoferrins as an iron-chelating agents with antimicrobial activity.[120] Transferrin receptors are involved in receptor-mediated transport and using their ligand can increase cellular uptake. When adsorbed at the surface of PLGA NPs, these surface modifications increased NtBD and the therapeutic effects of the drug compared to NPs without surface modifications.[121]

Lactoferrin receptors are highly expressed at the apical surface of respiratory epithelial cells, as well as neurons, especially neurons associated with age-related neurodegenerative diseases.[122] Using these receptors to penetrate neurons can be a way to improve treatments.[123] Pan *et al.* proved that after INA, lactoferrin-modified NPs were more abundant in the whole brain, than NPs without lactoferrin. The authors also demonstrated that lactoferrin minimize toxicity without reducing NPs bioavailability.[124]

Rabies virus glycoprotein (RVG) can also be used as a targeting ligand for NtBD. It is a protein responsible for neurotrophy following rabies virus infection. This glycoprotein can bind to acetylcholine receptors present at the neural cells' surface. Therefore, peptides derived from this glycoprotein are considered as targeting ligands for NtBD.[125] RVG29 is a peptide composed of 29 amino acids, a homologous segment of the RVG, used to target the brain. Polymeric NPs have been functionalized with this RVG29 to increase their uptake in the brain.[126] INA of RVG29-PEG-PLGA NPs led to a higher concentration of NPs in the olfactory bulb and the brain after 2 h.[127] Similarly, Chung *et al.*, who performed INA of PLGA-based NPs coated with RVG29, demonstrated that 2 h after administration, NPs coated with RVG29 were significantly more present in the olfactory bulb and the brain.[128]

And finally, another targeting ligand used can be borneol, a lipophilic terpenoid used in Traditional Chinese Medicine to enhance the delivery of other molecules to the brain.[129] NPs composed of PEG and PLGA were modified with borneol. After INA, borneol modified NPs were more effective in terms of treatment than NPs without borneol suggesting enhancement of NtBD.[130] In addition, INA of modified NPs with borneol were performed in rats and showed a higher brain accumulation compared to NPs without borneol.[131]

To conclude, using different molecules, from surfactants to CPPs, can be of great interest to functionalise the polymeric core of NPs and enhance their biodistribution in the brain.

4. Physicochemical characteristics

After considering the NPs composition for NtBD, we will now describe the NPs physicochemical characteristics, focusing on size and surface charge, as it strongly impacts their pharmacokinetics and pharmacodynamics properties. All data found are presented in Table 1 and Fig. 5.

4.1. Size

Cellular uptake, biodistribution and release kinetic of NPs are impacted by their size.[132] NPs under 200 nm are considered as a sort of virus and can be internalized by receptor-mediated endocytosis. These smaller NPs are more likely reach the nasal mucosa,[23] and it has also been shown that they ensure a better and faster crossing of the olfactory mucosa.[133] On the contrary, NPs above 500 nm should not be able to pass into the mucus

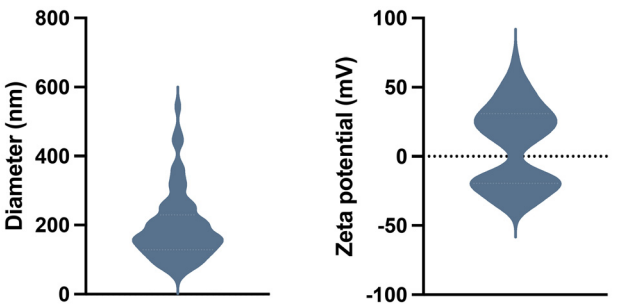

Fig. 5 Characteristics of NPs used for NtBD in terms of diameter (A) and charge (B).

pores as the pore size is around 150 nm.[134] These considerations need to be taken into account, but different sizes of NPs can lead to an efficient NtBD.

Several authors showed that NPs under 100 nm lead to a successful NtBD: Kanazawa *et al.* synthesized 80 nm PEG-PCL NPs;[135] Bhattamisra *et al.* optimized 73 nm chitosan NPs;[58] Veronesi *et al.* used 97 nm PLA-DPSE NPs;[136] and finally Seju *et al.* used 91 nm PLGA NPs.[137] All authors showed that their formulation was successfully reaching the brain after INA. It was shown that smaller NPs tend to diffuse faster in the mucus pores resulting in a more abundant brain accumulation for the smaller NPs.[134] It was also demonstrated by Cruz *et al.* that smaller PLGA NPs, around 100 nm, have a greater brain penetration than 200 nm PLGA NPs and even greater than 800 nm PLGA NPs.[138]

On the other hand, authors using polymeric NPs over 300 nm in size showed either a better efficacy or a higher concentration in the brain after INA: Guo *et al.* prepared 440 nm chitosan NPs;[139] Jain *et al.* used 351 nm PLA NPs;[70] Alex *et al.* developed 312 nm PCL NPs;[74] Pan *et al.* used 382 nm PEG-PLA NPs modified with lactoferrin.[124] The relation between mucus pore and NPs size was not discussed in these studies. Perhaps chitosan NPs, that are often larger than other types of NPs and that are positively charged, interact with mucin and therefore can moved into the mucus, getting closer to the epithelium. No other permeation enhancer was used, except for lactoferrin.[124]

This ability of reaching the brain no matter the size can be explained by the differential transport pathways depending on the NPs size.[140] It has also been mentioned that NPs larger than 100 nm (and under 200 nm) are more likely to be internalized *via* a clathrin-dependant endocytosis. Also, smaller NPs (60–90 nm) can enter the cell thanks to caveolin-coated endosome and NPs above these sizes can be internalized by micropinocytosis or phagocytosis.[141] Thus, size is not the only characteristics that need to be investigated, surface charges also need to be assessed as it will impact the affinity of the NPs with the mucus and the nasal mucosa.

4.2. Charge

Positively charged NPs have a limited permeability through the negatively charged mucin layer of the nasal mucosa.[17] They also have stronger interactions with mucus and can use the trigeminal pathway to reach the brain. Both limited permeability and strong mucus interactions slow their way to the brain. Several groups hypothesized that negatively charge NPs do not use an intraneuronal pathway therefore reaching the brain more quickly. But even if this theory seems interesting, there is no strong evidence other than the timing to support this hypothesis.[68,134,142]

Bonaccorso *et al.* compared different NPs: PLGA NPs that were negatively charged and chitosan/PLGA NPs that were positively charged. They showed that both NPs were able to reach the brain but with some slight differences. Negative NPs seem to be transported *via* the olfactory pathway whereas positive NPs were reaching the brain thanks to the trigeminal pathway.[143] Muntimadugu *et al.* synthesized PLGA NPs and lipidic NPs both negatively charged, these NPs were able to reach the brain.[144] Rukmangathen *et al.* used positively charged chitosan NPs that were found to have a pharmacological activity after INA.[145]

Finally, despite the surface charge being an important factor to explain nasal mucosa crossing, it is not the only factor that influence endocytosis and intracellular trafficking as there are a lot of different interactions between NPs and cells, and not all of them are based on the NPs surface charges. Indeed, cell membrane composition, homeostasis, and biomolecular corona that can happen around the NPs can all impact the mucosal penetration.

5. Nose-to-brain delivery evaluation

5.1. *In vitro* evaluation

NPs physicochemical characteristics significantly influence their biological properties, including cellular uptake and potential toxicity.[141] Although general assessments of toxicity and cell viability are not specific to nasal administration, this review emphasizes cellular models and methods that assess NP internalization, providing insight into their behaviour in the context of targeted nasal administration.

Several cell types and models are used to predict the impact of a new formulation on the nasal mucosa. These cells are mainly epithelial cells and neuronal cells. As seen in Fig. 6, these cells can either be cultivated on their own in a 2D or a 3D culture or with other cell types to create co-culture models.

5.1.1. Cell types. Different epithelial cells can be used to perform *in vitro* assays to demonstrate the relevance of formulations for NtBD. The most common cell type used for nasal metabolism as well as cell viability assays is the human RPMI 2650 nasal epithelial cell line.[146] Despite originating from human lungs tissue, Calu-3 cells have also been studied and are frequently used to evaluate formulations targeting the NtBD pathway.[147] Human bronchial epithelial 16HBE cells are also interesting because they have demonstrated *in vitro–in vivo* correlations.[147] Finally, primary culture of human nasal epithelial cells (HNEpC) expressing microvilli and mucin granule can be used.[148] These cells seem to be more relevant to evaluate NtBD formulations, but there are several drawbacks using them including their availability, difficulty of extraction, and short-term viability.[149]

Primary OSN could be a powerful model but they are difficult to harvest and to culture. Conversely, the rat pheochromocytoma PC12 cell line is one of the most common neuronal precursor cell lines in neuroscience research. When in contact with neuronal growth factor, these cells differentiate and express morphological and functional characteristics of neuronal cells.[150] Moreover, this cell line can be used to mimic neuronal disorders such as AD by administrating β-amyloid peptides to induce apoptosis and test new AD therapeutics.[151] Olfactory ensheathing cells, isolated from rat olfactory bulbs, were cultivated to evaluate formulations.[68]

Even though assessing the effect of NPs onto cells close to the olfactory mucosa cells is essential to the development of

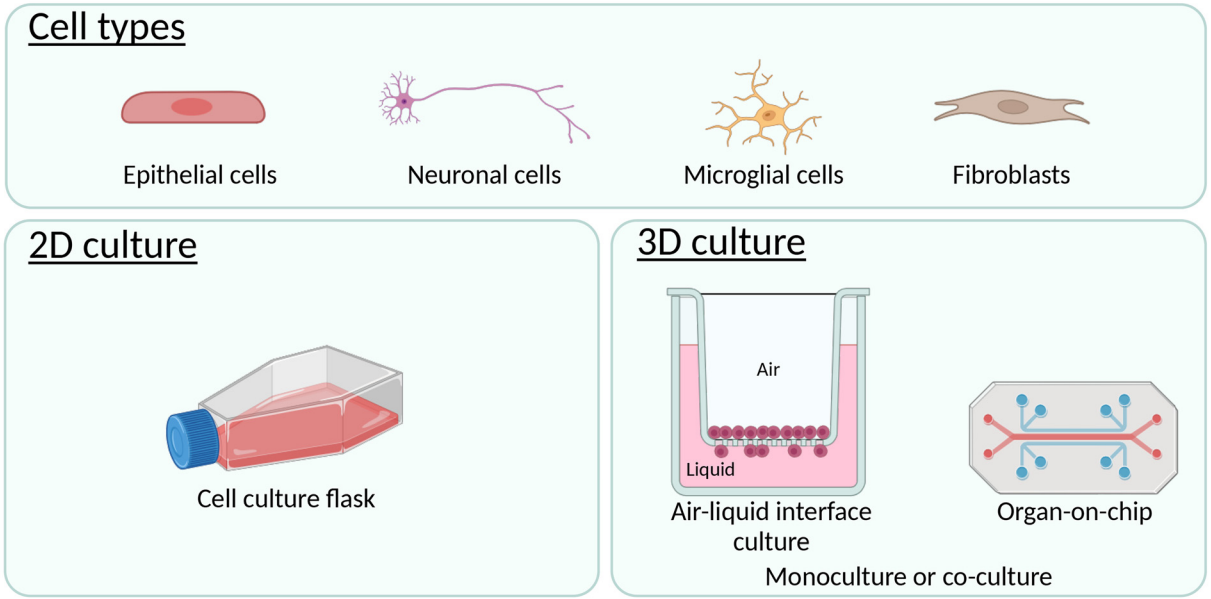

Fig. 6 *In vitro* evaluation of formulation for the NtBD. Created with BioRender.com.

brain targeting therapies, NP evaluation can also be performed on brain cells. For example, mouse microglial cells BV-2[87,152] were successfully used for the study of microglial biology in AD.[153] Human astrocytes, isolated from a human brain cortex,[148] were also used, not to assess the effect of the formulation on the olfactory mucosa, but for cytotoxicity assessment deeper in the brain. Foetal rat hippocampal neurons can also provide information on toxicity of bioactivity in *in vitro* experiments.[69]

These different cell types, used for 2D cultures, enable the composition of the nasal cavity to be imitated. For a more accurate representation of the nasal environment, co-culture systems or 3D models can be used.

5.1.2. Relevant *in vitro* models and co-cultures. In addition to 2D usual cell cultures, air–liquid interface (ALI) cultures can be carried out. Briefly, cells are seeded onto cell culture insert with liquid in both the apical and the basal compartment. After several days of culture, the apical medium is removed, leaving the culture in direct contact with the air (Fig. 6). This technique allows some cell types to differentiate and form TJs.[149]

Nasal epithelial cells RPMI 2650 can be cultivated onto cell culture inserts, forming a monolayer which integrity can be assessed using the transepithelial electrical resistance (TEER). It has been proved that the electric resistance is the same for RPMI cultivated at the ALI that the one from excised mucosa.[154] This ALI culture is used to get closer to the physiological condition, compared to 2D models, in which the nasal mucosa is in contact with the air breathed in and out. Plus, it has been mentioned that ALI cultures allow RPMI 2650 to form more TJs than cultivating them only in liquid.[155] Using fluorescent NPs, Schlachet *et al.* were able to determine the amount of NPs that crossed the RPMI 2650 monolayer overtime.[148]

This 3D cell culture model can be complexified by adding other cell types. Gabold *et al.* cultivated RPMI 2650 at the ALI and added U87 glioblastoma cells at the bottom of the plate containing the cell inserts. NPs were added onto this co-culture model to evaluate the cellular uptake using flow cytometry. The authors demonstrated the differential internalisation between the two cell types and showed a higher internalisation rate by epithelial cells compared to cancerous cells.[156]

With the recent development of organ-on-chips, Gholizadeh *et al.* have worked on a nasal mucosa-on-a-chip to recreate more physiological conditions using RPMI 2650. Evaluation of this new device showed that there was a differentiation into mucus-producing cells and a barrier function similar to the one of human nasal mucosa. Despite having numerous advantages, in this study, this organ-on-chip is still composed of one cell type, epithelial cells.[157]

Commercialized *in vitro* model can also be used, such as EpiNasal™ from MatTek. This model is an air–liquid interface culture of human nasal epithelial cells that expressed cilia and produce mucin thanks to goblet cells. This model has already been used to evaluate chitosan-PLGA NPs for nose-to-brain delivery.[158] Another 3D model on the market, MucilAir™ from Epithelix, is composed of basal cells, goblet cells and ciliated cells. Similarly, it is cultivated at the air–liquid interface, expressed cilia and tight junctions, and produce mucus.[159] Both these commercialized models lack an important cell type of the olfactory mucosa which is OSNs. This is probably due to the complexity of primary OSNs culture, maybe because of a specific microenvironment and difficulty of obtaining such primary cells.

These cell models are used to evaluate cell viability using assays such as the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay that evaluates metabolic

activity of cells[160] or the Sulforhodamine B assay that can be used of fixed cells.[161] Cellular uptake is also studied using cell models by using labelled-NPs and microscopy and/or flow cytometry.[148]

Despite having many different cell types and different culture methods, as well as relevant techniques for both cell viability and cellular uptake, the gap between *in vitro* evaluation and *ex vivo* is quite deep. To conclude, even if different cell types such as epithelial and neuronal cells can be used for both viability studies and cellular uptake determination, there is still a lack of a real olfactory mucosa model that could replace the use of animals and animal tissue.

5.2. *Ex vivo* evaluation

The use of tissue explants for the evaluation of the NP penetration through the olfactory mucosa is an improvement from using only cells, as it better mimics the native physicochemical environment and the coexistence of several cell types that are unavailable as primary cell lines. Therefore, it is considered that *ex vivo* evaluation is a more relevant model to evaluate new formulations. Besides, despite their animal origin, several explants can be sampled by excision from one single animal, then drastically reducing the number of animals used in a proof-of-concept study.[162] Although being relevant, these models still have some limitations due to the thickness of the mucosa and the lack of liquid flow.[146] In this chapter, we will detail the different types of *ex vivo* evaluation, including diffusion cell, nasal mucosa penetration evaluation and its integrity as shown in Fig. 7.

5.2.1. *Ex vivo* models. Excised nasal mucosa from animals is used in a large majority of cases,[163] as it is extremely difficult to obtain such tissue from human sources after surgeries. The most used is the goat nasal mucosa[20,164–171] obtained by post-mortem excision. It has been reported that the morphology of the ovine mucosa is more comparable to human nasal mucosa compared to other species. This is mainly due to the presence of ciliated and non-ciliated cells, basal and goblet cells, but also serous glands.[172] Sheep nasal mucosa are also largely used.[137,173–175] Other authors used rabbit,[84,124,176] porcine, bovine[180,181] or camel nasal mucosa.[182] Epithelium integrity can be verified by measuring the TEER, used as a marker of the permeability of the tissue.[183] To be noted that these experiments can be performed on excised mucosa after *in vivo* evaluation.

5.2.2. Diffusion cell. Various devices can be used to study excised mucous membranes. Among them, the diffusion cell represents a relevant device. A diffusion cell is composed of three main parts: the donor compartment where the formulation studied is applied; the receptor compartment containing a fluid; and in between, an epithelial tissue that can be skin or nasal mucosa in the context of NtBD evaluation.[184] This type of device can be used to evaluate both the receptor fluid and the mucosa after contact with the formulation.

To mimic the physiological condition of the nasal epithelium, the receptor fluid must have certain properties in terms of pH, temperature, and electrolyte composition. First of all, it has been reported that the pH in the human nasal mucosa is around 6.5.[185] In terms of temperature, it has been mentioned that the temperature in the region of the middle turbinate was of 32.3 °C.[186] Plus, the nasal mucosa is exposed to the air at the apical pole but is in contact with extracellular matrix and interstitial fluid at the basal side. A way to mimic

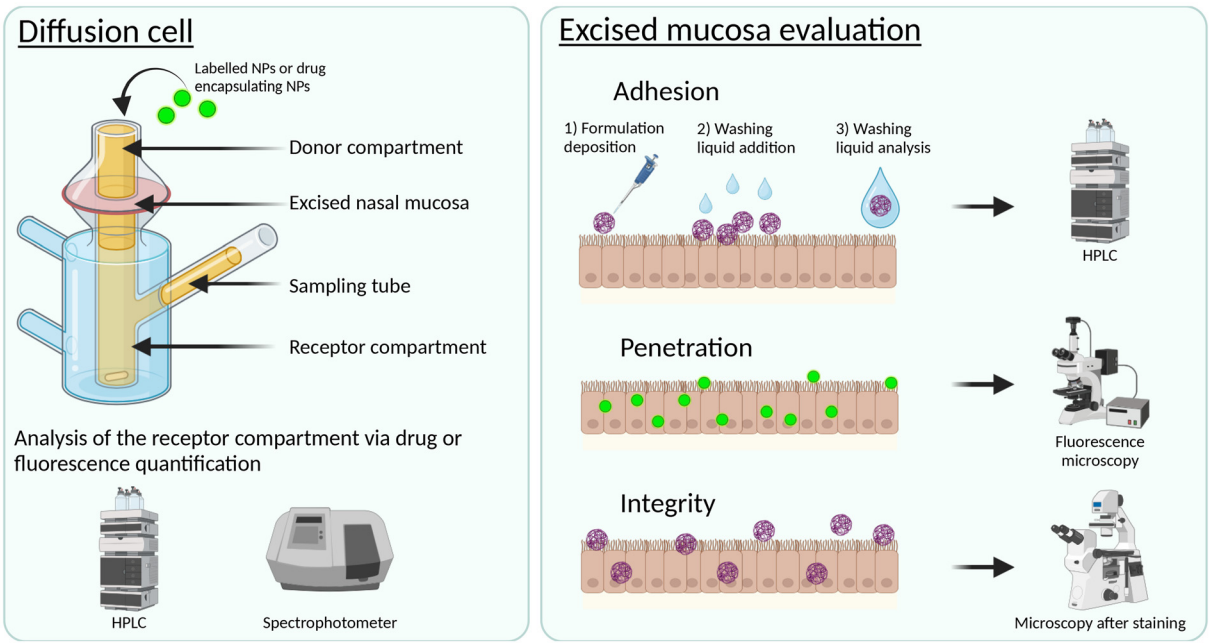

Fig. 7 *Ex vivo* evaluation of formulations for NtBD. Diffusion cell can be used to evaluate NPs permeation. Adhesion, penetration and nasal mucosa integrity can be evaluated using different methods, either directly on the mucosa or using a diffusion cell. Created with BioRender.com.

fluid movement in the basal compartment is to recreate a liquid that is close to the interstitial fluid. This fluid, especially the one that provides an brain-like environment, is composed of potassium, calcium, sodium and chlore ions.[187]

Several receptor fluids have been used. The more frequently used is the phosphate-buffered saline (PBS) solution that has a pH of 7.4 and is used at 37 °C.[165,166,168,169,176,182] Other types of electrolyte solutions can be used. Okmen Altas *et al.* and Clementino *et al.* both simulated a nasal electrolyte solution using NaCl, KCl and $CaCl_2$ at pH 6.5, and used it as a receptor compartment at 37 °C.[84,180] Pan *et al.* used an artificial nasal electrolyte solution composed of potassium, sodium and calcium ions at pH 6 and 34 °C.[124]

Once the receptor compartment filled and the nasal mucosa place above it, the formulation to be tested should be placed in the donor compartment, onto the excised nasal mucosa. At different time points, a small volume of the receptor fluid can be sampled. This sample can be analysed, and the passage through the excised mucosa. This analysis can be performed by dosing the drug in itself, by HPLC for example.[166] The main goal of using a diffusion cell is to obtain information on the drug passage of the mucosa. These studies are performed over time to have further information on the rapidity of this crossing.

5.2.3. Nasal mucosa adhesion and penetration. Adhesion properties of formulations can be evaluated directly onto excised nasal mucosa, by placing the formulation onto the mucosa and washing it. The washing liquid can be analysed to indirectly determine the amount of NPs or drug that adhered to the nasal mucosa.[84] This technique can also be used to assess the residence time of the formulation to estimate its resistance to mucociliary clearance. It was shown that PCL NPs were more adherent to the excised mucosa than the free drug they contained, meaning that it was not washed and could therefore cross the mucosa in larger quantities.[75]

The penetrating capacity of NPs can also be evaluated using confocal microscopy and fluorescent dyes. In this case, NPs that stayed in the mucosa can be analysed, whereas in the diffusion cell, NPs that cross the mucosa are analysed, these two experiments being compatible with each other. Nasal mucosa can be fixed, sliced and observed using microscopy to visualise the localisation of the NPs within the tissue.[61,70] Using excised nasal mucosa to evaluate NPs penetration in the tissue are a relevant way of getting information on the localisation of the NPs in the cells and to have a more robust model compared to 3D cell culture that can be less representative as they don't, for the moment, include all the different cell types in the cell culture.

5.2.4. Nasal mucosa integrity. After evaluation of the formulation mucosal permeability, excised nasal mucosa can also be used to evaluate the nasal mucosa integrity. This can be performed by sectioning and staining it with haematoxylin and eosin. Histological changes in the epithelium can be observed, especially superficial desquamation, inflammation, epithelial disruption or cell necrosis, or even damage of the cilia.[164]

To conclude, *ex vivo* evaluation offers the use of a more robust model to evaluation diffusion through the nasal mucosa as well as adhesion and tissue integrity. The next step of evaluating NPs for NtBD is *in vivo* evaluation, with all the limitations of the nasal cavity, mucus, mucociliary clearance, and enzymes.

5.3. *In vivo* evaluation

In vivo evaluation in animals is essential before any clinical trials. The choice of the animal models, the administration method, volume and protocol are determined according to the disease to be treated as well as the results expected. Both pharmacokinetics (PK) and pharmacodynamics (PD) can be evaluated to first follow the fate of the drug in the body and secondly to evaluate the drug activity on the disease treatment. In this chapter, we will go into further details into the *in vivo* methods to evaluate NtBD of therapeutic polymeric NPs.

5.3.1. Animal models and anatomy of the nasal cavity. *In vivo* evaluation is necessary to complete the formulation analysis as it allows the determination of PK and PD characteristics as shown in Fig. 8. The anatomy of the nasal cavity is crucial when choosing an *in vivo* model because of the differences that remain between human and animals. For example, rodents present atrioturbinates, a vomeronasal organ, Steno's glands, and a septal window that are absent in human adults. These differences modify the airflow pathway upward and lateral in the nasal cavity, towards the olfactory mucosa, instead of downward to the nasopharynx like in humans potentially misleading analysis of NPs pathways. Moreover, 20% of the air inhaled passes through the olfactory region in rodents, whereas this number drops to 3% in humans.[188,189] Finally, the olfactory region represents 50% of the surface of the nasal cavity in rodents whereas it is 10% for humans.[146] Therefore, it might be easier for NPs to take direct olfactory pathway in rodents. However, they are not that costly, easy to handle, and they can provide relevant data to study biological processes and drugs' efficacy *in vivo*. Therefore, rats and mice are the most used animal model for the NtBD[190] even if monkeys seem closer to humans anatomically speaking.[188]

5.3.2. Administration. When administrating drugs to rodents for the NtBD evaluation, different characteristics must be considered: the volume of administration, the consciousness of the animals and the devices used to administer the product.

5.3.2.1. Administration volume. Even if the volume of the rat nasal cavity is 260 μL to 400 μL (ref. 189) and for mice 32 μL,[191] different volumes of administration are documented in the literature. Most authors chose 20 μL (ref. 106, 174, 175, 192 and 193) or 100 μL (ref. 130, 135 and 194) even if volumes vary between studies from 10 μL (ref. 195) to 200 μL.[65] These volumes are often distributed between the two nostrils. For both rats and mice, little explanation is provided concerning the choice of such volume, or the repetition of administrations of smaller injection volume when the volume mentioned is large. According to the Institutional Animal Care and Use Committee of San Francisco University, United States, the rec-

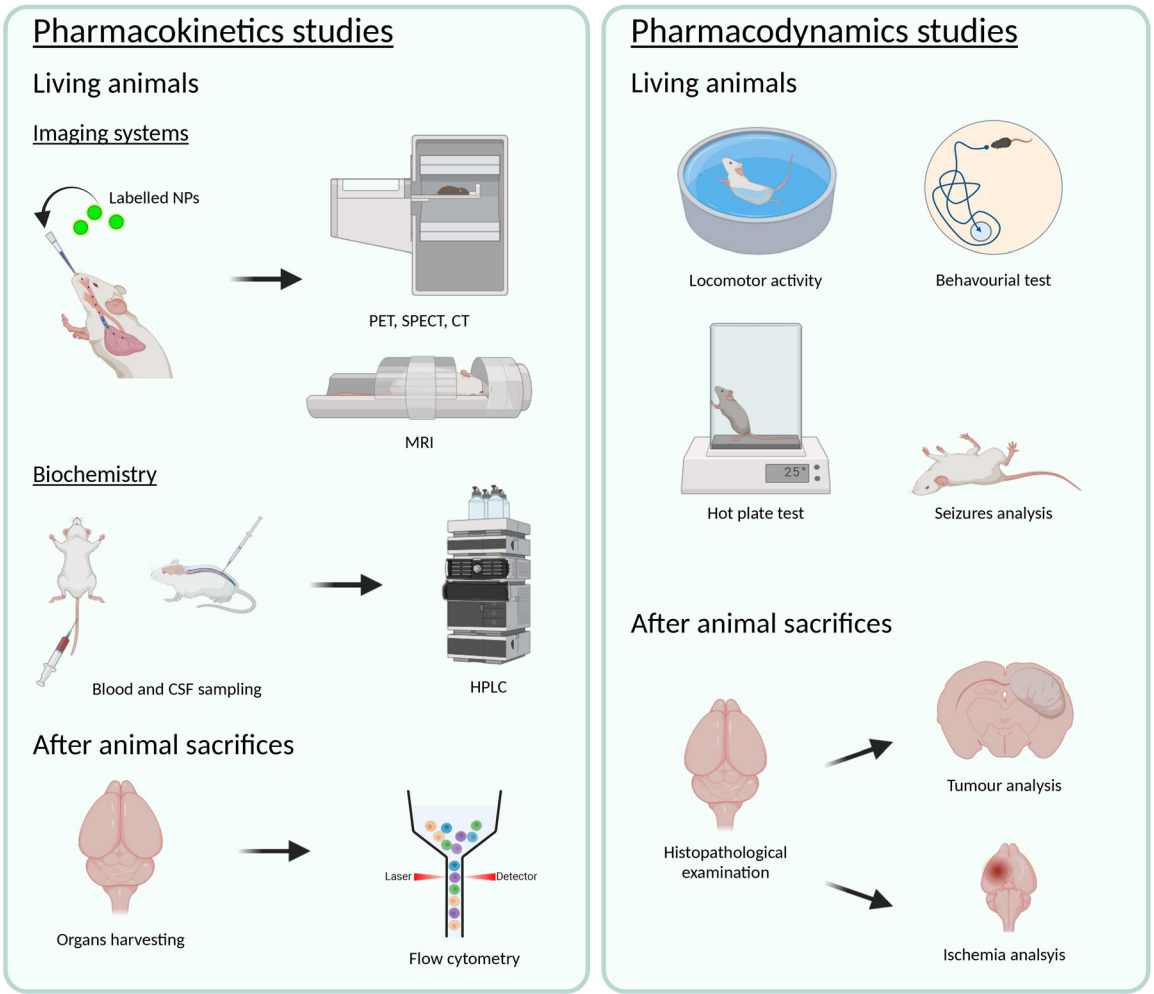

Fig. 8 *In vivo* evaluation of formulations for the NtBD. Created with BioRender.com.

ommended volume for nasal administration is below 50 μL for mice and around 100 μL for rats.[196]

However, the field would greatly benefit from more homogeneity in the protocols of INA of liquid formulations. The volume administered is indeed of most importance because an excess of liquid can cause the animals to swallow and lead to loss of formulation that does not reach the olfactory mucosa. Plus, this swallowing can induce an experimental bias due to the use of the oral route as well as the nasal route.

Additionally, studies have shown that instilled volume in the nose can impact the distribution of the formulation in the nasal cavity.[197] It was demonstrated that the bigger the volume, the more chance there are that the product reach the lungs and not just the olfactory region of the nasal cavity.[197]

5.3.2.2. Anesthesia. Formulations can be administered on conscious animals.[198] But usually, anaesthesia is performed by intraperitoneal injection of drugs such as pentobarbital[135] or ketamine/xylazine.[75] It can also be performed using inhalation anaesthetics such as isoflurane.[199] Similarly to the volume administered, there are many differences of anaesthesia between studies, however, there is not any difference in terms of organs distribution.[197] The type of anaesthesia has no impact on the formulation distribution in the nasal cavity.[197]

5.3.2.3. Administration devices for nasal delivery. Different devices can be used to administer products in the rodent nose. The most used is the micropipette technique, where drops are deposit at the entrance of the nose. These drops are then inhaled during normal breathing of the animal[59,200] place in supine position.[146] The main advantages of this technique are the non-invasiveness and the inhalation that is very close to what can happen in humans. But, if the volume is too large, or the animal is conscious, it can lead to swallowing, loss of formulation in the digestive tract and bias of administration.

A region-targeted device can be useful to maximise the contact between the formulation and the olfactory mucosa. To this end, several authors have been using a catheter attached to a micropipette or a syringe to administer a smaller volume very locally.[139,144] This technique was proven to limit the risk of swallowing or lung deposition.[201] Nevertheless, micropipette administration seems less invasive and less damaging for the nasal mucosa than inserting a tube in the rodent nostril.[202]

Once again, differences between studies underline the lack of consensus for INA in NtBD. These discrepancies can have an impact on the heterogeneity observed in terms of retention time in the nasal cavity or even concentration of drug reaching the brain.[203]

These administration techniques are adapted from human nasal administration using different devices. These devices can be nasal drops or nasal sprays.[204] Nasal drops, similarly to micropipette administration in rodents, require a dropper that is inserted in the nostril to administer drops in the nasal cavity. The main advantage of this technique is the improved deposition of the drug in the nasal tract and the lack of preservatives. Despite that, this device is not very patient-friendly as the administration requires a head tilted back and neck extended which can be very uncomfortable for elderly patients.[47] Nasal sprays, contrary to nasal drops, allow a better measurement of the dose administered. They are very much easier for patient self-administration and offer better patient compliance, plus, they are low-cost and can be easily manufactured. Therefore, nasal sprays are the most-used device for INA in humans.[47,204]

The different methods used for nasal administration in animal models show significant heterogeneity, reflecting differences in techniques, volumes administered and delivery devices that may influence the results and comparability of studies.

5.3.3. Toxicity evaluation. Besides completing some PK and PD studies, experiments were also performed to evaluate the toxicity of formulations *in vivo*. For example, either PCL[75] or chitosan[205] NPs were administered to the animals, these NPs could have been blank or loaded ones. The nasal mucosa was observed post-mortem after haematoxylin and eosin staining. They looked for epithelial disruptions, extracellular debris presence, cell and cilia structures, similarly to what was described in section 5.2.4. (Nasal mucosa integrity). The administration could have occurred several times before any observations[75] or just once.[205] Inflammation markers analysis could have been performed to obtain more accurate information on inflammation of the nasal mucosa after INA of NPs, for example, interferon or interleukin expression could be evaluated.[206]

5.3.4. Pharmacokinetics studies. Finally, pharmacokinetics (PK) assess the distribution of the drug within the whole body, from administration to elimination, as well as its residence time in the body.[207] To evaluate the PK of NPs after INA, several techniques can be used on living animals or after animals' euthanasia.

5.3.4.1. On living animals. The use of imaging to assess new formulations for NtBD has been an interesting tool, as NPs can be used as imaging agents. To that end, several techniques can be used: magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), single-photon computed tomography (SPECT) and fluorescence tomography. Even though MRI allows a huge spatial resolution as well as a relevant sensitivity, it remains quite costly. SPECT and PET offer an important sensitivity but are also quite expensive.[208] Moreover, with PET imaging, due to the short distance between the nostrils and the brain, it is difficult to quantify the contrast agent in the brain. Finally, CT can provide a strong spatial resolution and is relatively inexpensive.

Fluorescence tomography is the most commonly used modality, thanks to several advantages including its low cost, its easiness of use, its high sensitivity, and the fact that signal is obtained very fast. One main drawback is the low penetration capacity and the presence of autofluorescence noise at certain wavelength.[201] Different solutions exist to avoid this background fluorescence including the choice of wavelength in the near-infrared region (above 750 nm) and the use of a chlorophyl purified diet.[209]

Different fluorescent probes can be used. For example, DIR is a fluorescent cyanine dye that is excited at 748 nm and emit at 780 nm that can be easily encapsulated into NPs due to its lipophilia. It has been the most common fluorescent probes for biodistribution studies. These authors compared their formulations with either the free fluorescent probe, or their formulation without the surface functionalisation,[122,210] or to choose between their different formulations,[64,127] they also used this technique to compared administration patterns.[194]

Other authors used cyanine-based probe Cy5.5, that is excited at 675 nm and emit at 694 nm to image organs or perform blood and organs dosing to prove the advantage of lactoferrin surface modifications of their NPs.[106] IR-780 dye, which is a iodide dye that is excited at 780 nm and emit at 799 nm can also be used to image mice by fluorescence tomography.[211]

To overcome the drawbacks of imaging modalities on their own, an innovative solution is to use combined images from different modalities. These combined techniques can be PET/CT, like Veronesi *et al.* who used the latter to obtain brain images with radiolabelled NPs with zirconium (^{89}Zr). Nevertheless, they also mentioned the difficulty to obtain subregional cerebral localization of the NPs.[136] SPECT uses the same radiolabelled NPs but the resolution is lower than MRI or CT. This technique, mainly due to its rapidity, is still used to evaluate the fate of radiolabelled NPs after INA. The contrast agent often used is the technetium (^{99m}Tc). Images can be obtained at different time points to evaluate the kinetic evolution of the NPs. With this technique, authors showed that INA of NPs led to a stronger signal in the brain than when NPs were administered intravenously or when compared to the free contrast agent.[35,174,212,213]

The main advantage of using imaging techniques, alone or combined, on living animals is the easiness of following the NPs over time in the animal body. But the main drawback of these techniques is the lack of precise information on location. Due to the nasal anatomy and the proximity between the nasal cavity and the olfactory bulb, it can often be difficult to evaluate whether NPs are still in the nasal cavity or in the olfactory bulb. But information on elimination of the NPs from the head or loss of formulation in the digestive track can be followed up. Imaging organs more closely or dosing the contrast

agents in the brain or the blood can be a complement to obtain this location information.

5.3.4.2. Ex vivo. As mentioned earlier, imaging agents can be easily encapsulated into NPs to perform further analysis. After INA of NPs using radiolabels or fluorescent dyes, blood samples can be taken and organs, including brains, can harvested. Radioactivity was then evaluated in all these samples.[35,192,195,212] Brains have also been dissected and radioactivity was measured in different parts of the brain such as the olfactory bulb, the brain stem and the forebrain.[136] This can also be an advantage to use this technique before and after euthanasia.

Using fluorescent NPs, containing rhodamine B or curcumin for example, organs were then analysed by fluorescence microscopy.[64,87,152,170] After brain collection, sections were analysed by immunofluorescence for refined drug localization analysis.[65] Fluorescence was also just quantified in full organs[106,109,214,215] or in homogenized brains.[135] Organ tissues were also analysed using flow cytometry to evaluate the cell proportion having internalized the fluorescent NPs.[216]

Another technique used is drug dosing either by high performance liquid chromatography (HPLC) or mass spectrometry. Drug dosing can be performed in the blood, in the brain and in other organs, at different times points and at the end of the study.[87,139,156] Drug dosing was also performed in the CSF.[217]

To conclude, evaluation of the NPs PK profile on living animals is made easy by the possibility of encapsulating an imaging agent. Different imagining systems can be used to obtain information on NPs localisation and sometimes, NPs concentration, being in itself one of the main advantages of using polymeric NPs. This PK evaluation can be performed by simple drug dosing in the blood using HPLC or mass spectrometry. After animals' death, further information can be obtained by using organs for drug dosing or microscopy. Despite having information on localisation and/or drug concentration in different parts on the body, there is one remaining question, and that is the effect of the NPs on the body.

5.3.5. Pharmacodynamics evaluation. Pharmacodynamic (PD) studies are essential for evaluating the effects of a drug on the body and are typically conducted using animal models. These experiments are carefully tailored to align with the specific pathology under investigation and the therapeutic outcomes or mechanisms of interest. This customized approach ensures that the unique aspects of each disease and the intended drug actions are accurately represented, providing critical insights into drug efficacy and optimizing treatment strategies. In this section, we will explore key criteria commonly assessed in the context of brain pathologies.

In brain tumours, Kanazawa *et al.* administered their PEG-PCL NPs functionalized with the Tat CPP and estimated the tumour growth on brain sections.[135] Chu *et al.* administered their PLGA NPs and counted the number on apoptotic cancerous cells in brain sections.[64]

To evaluate the efficacy of a pain medication based on chitosan NPs, different tests have been performed onto animals such as a hot plate test, or a writhing test after INA.[169,177] To study a migraine treatment composed of chitosan NPs, the measurement of photophobia, abdominal stretching and constriction was performed.[218]

To study neurodegenerative diseases, behavioural tests and histopathological examinations are often performed. In Parkinson's Disease, biomarkers measurements in brain tissue can be performed.[58,166,219] AD therapy have also been assessed by visualising the neuroprotective effect of the PEG-PCL NPs.[106]

Depression treatments have also been evaluated using different behavioural tests such as the swim test, or the sucrose preference test, after INA of chitosan-coated PLGA NPs;[101,182] chitosan NPs;[156,179] and PLGA NPs.[171] Antipsychotic activity have been determined to evaluate a schizophrenia treatment based on PCL NPs.[75]

In cerebral ischemia therapies, PEG-PLGA NPs[127,130,220] or chitosan NPs[221] have been evaluated by neurological assessment and multiple sclerosis chitosan NPs based-treatment by investigating the brain histopathology.[152] Evaluation of seizure appearance, as well as biochemical and histopathological evaluation using disease markers have been performed after INA of chitosan NPs.[194,200]

Finally, to evaluate the efficacy of an intracerebral haemorrhage treatment based on chitosan NPs, researchers evaluated the brain water content. They also performed histology and biochemistry studies to evaluate the neural injuries with and without treatment.[139]

The type of PD evaluation can also be dependent on the type of drug that is used. For example, siRNA was developed to treat Huntington's disease and encapsulated in chitosan NPs. Experiments of gene silencing in the brain using this siRNA were performed by qPCR to evaluate the potential of this siRNA onto Huntington's mice model.[222]

Besides showing the efficacy of the treatment on the specific disease targeted, evaluation of side effects and especially toxicity needs to be performed to ensure the treatment relevance and the possibility of switching to clinical evaluation.

To conclude, PD evaluation of NPs administered intranasally are as various as the diseases they're supposed to treat. Nevertheless, this evaluation is extremely relevant to conclude on the treatment efficacy. *In vivo* evaluation seems essential to demonstrated the relevance of any treatment. In the context of NtBD, PK and PD evaluation are complementary to demonstrated that not only NPs are located in the brain but that they can have an impact on the disease studied.

6. Safety

Polymeric NPs are generally regarded as safe and biocompatible. But even though NtBD seems a promising approach for drug delivery to the brain, several challenges remain. Physiological conditions can be disrupted by INA, resulting in altered pH or osmolarity, impacting mucociliary clearance.

Side effects such as congestion, dryness, and irritation can occur and are quite difficult to predict even using *in vivo* models.[223]

Plus, their chronic use raises concerns, especially about hypersensitivity reactions, that have already been demonstrated using PEG or polysorbate 80.[224] Looking forward, NPs safety could be addressed more systematically when developing new delivery systems based on polymeric NPs for the NtBD.

7. Clinical evaluation and future aspects

Finally, the last step of the development of NPs for the NtBD is the clinical translation. To date, no polymeric NPs for the NtBD are on the market or approved by the Food and Drug Administration.

At the time of this review, 96 clinical trials concerning NPs were active or recruiting patients. Among them, none mentioned intranasal administration and only 17 were related to the brain.

The absence of clinical trials evaluating polymeric NPs for NtBD is probably the result of a gap between preclinical evaluation and clinical application. This is due to the challenges in upscaling NPs synthesis under Good-Manufacturing Practices conditions.[225,226] Besides, regulation remained unclear and there are no harmonized criteria for nasal deposition, adsorption, and biodistribution. All these can prevent clinical translation to happen.[227]

Plus, there are a lot of anatomical differences between animals as mentioned earlier, but also interindividual variability exists in humans[228] resulting in complex targeting of the olfactory region.

Finally, clinical trials have been conducted using INA of biomolecules such as insulin to treat AD, but no NPs was involved. But it has been mentioned that using a drug delivery system can improve even more therapeutic effect, by increasing permeability and limiting clearance.[229]

To overcome these clinical challenges, better translational models need to be developed such as olfactory organoids[230] but also imaging systems that can monitor brain delivery in real time.[231]

8. Conclusions

Treatment of brain diseases is challenging largely due to the BBB. NtBD is a promising strategy to bypass this biological barrier. Due to the nasal anatomy, the olfactory mucosa, and the presence of pathways from the nose to the brain, INA has proven to be an efficient tool to obtain high drug bioavailability in the brain and to maintain drug efficacy. In addition to direct NtB pathway, INA has numerous advantages including minimizing the risk of side effects and being a painless and non-invasive administration route. Nevertheless, protection of the drug itself is essential due to mucus, mucociliary clearance or nasal enzymes and that is why polymeric NPs are a very promising strategy to vehicle the drug (drug protection, cell internalization, and optimized targeting to reach their goal). Therefore, optimizing the most effective NPs for crossing the different biological barriers is one of the main goals in designing a treatment for the NtB pathway.

In this review, the main characteristics for this design have been established: polymer choice from chitosan to PLA, functionalisation of the NPs to improve stealth or cell penetration, and physicochemical characteristics, mainly size and charge, to obtain an effective candidate for the NtBD. Information on the evaluation of the NPs were provided *in vitro* using cell cultures in two or three dimensions. New cell culture techniques such as organ-on-chips are developed to evaluate more accurately NtBD formulation. After *ex vivo* evaluation on excised nasal mucosa, *in vivo* experiments evaluating both PK and PD can be realised. Finally, polymeric NPs and NtBD can be used in numerous applications, including neurodegenerative diseases, depression or brain tumours, therefore having an important impact on public health matter.

Despite the growing interest and immense hopes for these innovative NPs, the nasal route of administration and promising *in vivo* results, there are currently no clinical trials. Also, there are still difficulties in transposing treatments for brain diseases that directly or indirectly affect a large proportion of the population, such as the choice of a relevant animal model or the lack of fully predictive *in vitro* models that could slow down clinical translation.

Abbreviations

AD	Alzheimer's disease
ALI	Air–liquid interface
Aβ	Beta-amyloid
BBB	Blood-brain barrier
CIRI	Cerebral ischemia and reperfusion injury
CPP	Cell-penetrating peptide
CSF	Cerebrospinal fluid
CT	Computed tomography
HPLC	High-performance liquid chromatography
INA	Intranasal administration
IV	Intravenous
LMW	Low molecular weight
MRI	Magnetic resonance imaging
MTT	3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NP	Nanoparticle
NtB	Nose-to-brain
NtBD	Nose-to-brain delivery
OSN	Olfactory sensory neuron
PBS	Phosphate buffered saline
PCL	Poly(ε-caprolactone)
PEG	Polyethylene glycol
PET	Positron emission tomography

PD	Pharmacodynamic
PK	Pharmacokinetics
PLA	Polylactic acid
PLGA	Poly(lactic-*co*-glycolic acid)
PVA	Polyvinyl alcohol
RVG	Rabies virus glycoprotein
SPECT	Single-photon computed tomography
TAT	Transactivator of transcription
TEER	Transepithelial electrical resistance
TJ	Tight junction
TPGC	D-α-Tocopherol polyethylene glycol 1000 succinate
UEA-I	Ulex europeus agglutinin I
WGA	Wheat-germ agglutinin

Author contributions

Conceptualization: SR and CM; data curation: MB; formal analysis: MB; funding acquisition: SR; investigation: MB; methodology: SR, MB, MF; project administration: SR, CM, MF; resources: CM, SR; supervision: SR, CM, MF; validation: SR, CM; visualization: MB; writing – original draft: MB; writing – review and editing: SR, CM, MF.

Conflicts of interest

There are no conflicts to declare.

Data availability

The authors confirmed that no primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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