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Tackling myelin deficits in neurodevelopmental disorders using drug delivery systems☆

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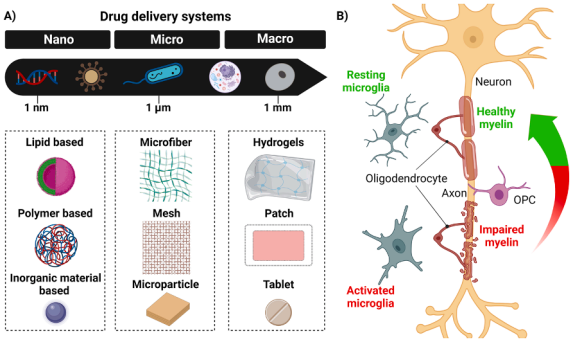
GRAPHICAL ABSTRACT



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ABSTRACT

Interest in myelin and its roles in almost all brain functions has been greatly increasing in recent years, leading to countless new studies on myelination, as a dominant process in the development of cognitive functions. Here, we explore the unique role myelin plays in the central nervous system and specifically discuss the results of altered myelination in neurodevelopmental disorders. We present parallel developmental trajectories involving myelination that correlate with the onset of cognitive impairment in neurodevelopmental disorders and discuss the key challenges in the treatment of these chronic disorders. Recent developments in drug repurposing and nano/micro particle-based therapies are reviewed as a possible pathway to circumvent some of the main hurdles associated with early intervention, including patient's adherence and compliance, side effects, relapse, and faster route to possible treatment of these disorders. The strategy of drug encapsulation overcomes drug solubility and metabolism, with the possibility of drug targeting to a specific compartment, reducing side effects upon systemic administration.

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1. Introduction

Myelin, the lipid substance engulfing the axons of the central and peripheral nervous systems (CNS and PNS, respectively), is a highly conserved structure. It enables saltatory conduction, supports axonal integrity, enhances impulse conduction, and promotes brain wide synchronization [1]. Small variation in myelin thickness and structure allows for adaptive neural network functions [2,3]. Like any other structure, myelin is prone to be aberrantly developed or altered by genetic factors [4] or environmental conditions [5]. One class of disorders with pathologies involving myelin alterations is neurodevelopmental disorders (NDDs); a group of conditions associated with abnormalities of the neurological system and developmental deficits, which leads to behavioural alterations including difficulties with language acquisition, memory, learning and motor skills. These symptoms, originating in altered brain development, can be attributed, among other reasons, to maldeveloped white matter (WM) in the CNS.

The challenges to both the patients and their support system are immense. As NDDs often involve developmental disabilities, the patients require intense consistent care, with high financial cost for treatments and aids. Today, pharmaceutical therapies for NDDs are focused mostly on symptoms' relief rather than targeting the underlying causes. This is due to our lack of mechanistic understanding of the pathophysiology responsible for these disorders. Such understanding will enable the development of relevant drugs aiming to defined targets. However, the process of new drug development, its approval and eventually reaching the patients remain a time consuming and highly expensive path. To expedite this process, efforts are being made to repurpose known or approved drug molecules for the treatment of new indications. Several drug molecules have been identified as potent remyelination agents and their applicability is being investigated in ongoing clinical trials. Although this can lead to successful interventions, most of these approved drugs also suffer from some major drawbacks when being repurposed as a remyelination agent. For example, some molecules are extremely hydrophilic and undergo fast removal upon oral administration, leading to high and repeated administrations, while others suffer from low drug absorption and bioavailability upon oral administration. Instead, for parenteral intravenous *(iv)* injections the major problems involved are patients' compliance and lack of blood brain barrier (BBB) penetration of the molecules.

To address these issues, polymeric and lipidic nano and micro particle-based systems have emerged with significant promise in the development of formulations comprising of promyelinating drugs for the treatment of NDDs. While nanoparticles (NPs) can potentially assist in crossing the BBB, reducing drug related systemic side effects and drug metabolism, microparticles instead can ensure a steady drug concentration, decrease drug administration frequency, and increase patients' compliance. In view of these advantages, the current developments in the field of drug repurposing and the use of nano and micro particle-based therapies involving promyelination drugs are concisely reviewed in the current work.

2. Myelin

2.1. White matter components

Myelin is the insulating layer around nerves; it is a lipid-rich substance that gives the WM its fitted name. Myelin has a crucial role in impulse transmission along the neuronal axon [6], in both the CNS and PNS, with differences in structure, cellular players, developmental process and ratio of molecular components [7,8]. Briefly, while oligodendrocytes (OLs) are responsible for myelination of the CNS, where every cell can myelinate multiple segments (Fig. 1**A**), in the PNS myelination is provided by Schwann cells, each typically myelinates a single fiber [9,10]. As the focus of this review is myelin alterations in NDDs, a group of disorders in which behavioural deficits are prominent, originating in brain development deficits, we describe myelination of the CNS only, and the role WM plays in the development of neural circuits [9,11].

In the CNS, myelin is composed of ~75 % cholesterol and lipids of the phospholipids and glycolipids families [12,13], while the remaining ~25 % is composed of proteins such as proteolipid protein (PLP), myelin basic protein (MBP), 2,′3′-cyclic nucleotide 3′-phosphodiesterase (CNP) and others, which compact the sheaths of myelin membrane tightly together [14] and stabilize the myelin structure [15] (Fig. 1**B**). The contribution of myelin to axonal conduction is achieved by its segmentation across the axon; the myelin is not continuous, prompting unmyelinated areas, the Nodes of Ranvier (Fig. 1**C**), in which clustering of sodium channels in a small area occurs, enabling saltatory conduction of electrical signal with minimal consumption of energy [16].

OLs are the glial cells responsible for myelination of the CNS: they extend their plasma membrane to envelop certain axons [9] in tight layers (myelin sheaths), providing metabolic support [17], enabling fast nerve conduction [18] and maintaining axonal integrity [9] (Fig. 1**D**). Recent findings suggest that axonal integrity is even kept by OLs independently from processes of myelination [18,19].

The development of OLs is set in tightly regulated steps of the oligodendroglia lineage [20], starting with oligodendrocytes precursor cells (OPCs) that originate in three, functionally redundant, waves (in the medial ganglionic eminence and anterior entopeduncular area of the ventral forebrain, the lateral and caudal ganglionic eminences, and in the postnatal cortex) [21]. The OPCs then migrate across the brain, proliferate, and differentiate into pre-myelinating (immature) and myelinating OLs (mOLs) [22,23].

The continuous myelination across the rodent [23] and human [24] lifespan, including maturation of OPCs to mOLs [25], is carried out by the differentiation of existing OPCs, accounting for ~5–8 % of the cells in the adult brain of rodents [26–29] and humans [29] (Fig. 2**A**). Nearly all OPCs in the human brain are generated in the first 5 years of life [28] and play a role in remyelination in response to injury (by replacing the damaged OLs) [30,31] and in learning mechanisms that require the generation of new mOLs [32,33]. Purinergic receptors (activated by ATP) in the neuron-glia synapse regulate proliferation, differentiation and maturation of OPCs and OLs in response to neural activity [34,35], which leads to the selective myelination of unmyelinated or partially myelinated axons in the adult brain [36–38], increasing impulse propagation and information transmission [39] in specific circuits and fine-tuning the neural network. In previous work with mice for example, OPC proliferation and differentiation was crucial for motor skill learning [40,41] and memory [42].

Microglial cells, the tissue resident immune-system cells, are derived from myeloid precursors and play a critical role in mediating inflammation, contrasting pathogen infections, maintaining brain homeostasis, and removing cellular debris through the phagocytosis process. Besides their neuroimmune functions, they can modulate the synaptic formation, signal transmission, and neuronal activity, playing a crucial role during different stages of CNS development [43].

Astrocytes are the most abundant cells in the brain, deriving from a neuroepithelial precursor in common with neurons and OLs. They can modulate synaptic activity, neuronal functions, and brain homeostasis [44]. Although fundamentally different in origin and function, microglial cells and astrocytes can communicate with each other to regulate and control neurogenesis, axonal growth, synapses formation and pruning. These cell types have also been described to contribute to myelin production, modulating the endogenous capacity of OPCs to initiate remyelination [45]. They do so by producing and secreting different sets of trophic factors and cytokines, which can modulate OPC differentiation, OL genesis and maturation [46].

Glial cells dysfunction could lead to brain development and brain diseases with a late onset [47,48]. In pathological conditions, where an imbalance between demyelination and remyelination occurs, the clearance of myelin debris by microglial cells is critically important to

slow the pathogenesis and guaranty sufficient remyelination, an area of great interest in therapeutic research [49–52]. Microglial cells also participate in the OPC differentiation and maturation with the release of pro-regenerative factors [53]. Astrocytes stimulate the recruiting of microglia to the site of demyelination, produce regenerative mediators, and contribute free–circulating lipids to the damaged site, prompting the activated OPCs to migrate and differentiate, and favouring remyelination [54,55].

2.2. Myelination

Myelination is a non-simultaneous developmental process, beginning in posterior areas of the brain and continuing to the front [56,57], with different regions exhibiting typical myelination patterns, like fast onset - fast completion of the optic nerve and auditory brain stem, or onset followed by a pause and then continuous myelination in the cingulate and parahippocampal gyri [58]. In humans, the first steps of myelination are detected *in utero*; markers of OPCs can be identified as early as 5 gestational weeks (GW) [59], and active mOLs are present by 18–25 GW [59,60]. The process of myelination continues after birth [61,62] and follows a non-linear trend, with three distinct waves of substantial, synchronized myelin changes [63,64] (Fig. 2**B**), which include myelination of previously unmyelinated axons [65], alternations to internodal number and length [66,67], changes to myelin thickness [68] and changes to overall volume of WM in different regions [64,69]. These myelination waves differ in clear kinetics across the lifespan: the first wave occurs in early childhood, typically refers to as ages 3 months to 4 years [70], with rapid myelination of anterior and upper cortical areas [71] which correspond with development of cognitive functions [70,72]. The second wave during adolescence (10–20 years of age), includes prominent myelination of the grey matter, with inter-regional increase in myelin content generally associated with behavioural changes and skill acquisition [73,74]. During adolescence, myelin content in cortical areas as well as WM is correlated with puberty status [75]. The third myelination wave refers to the slow demyelination during aging [63]. It is important to note, that while distinct myelination peaks can be named, there are sub-waves that can be inspected within each peak [62] and changes in myelin of individual axons and brain regions still occur [63,64]. As myelination is mostly a postnatal process, normal myelin development is susceptible to environmental stimuli and

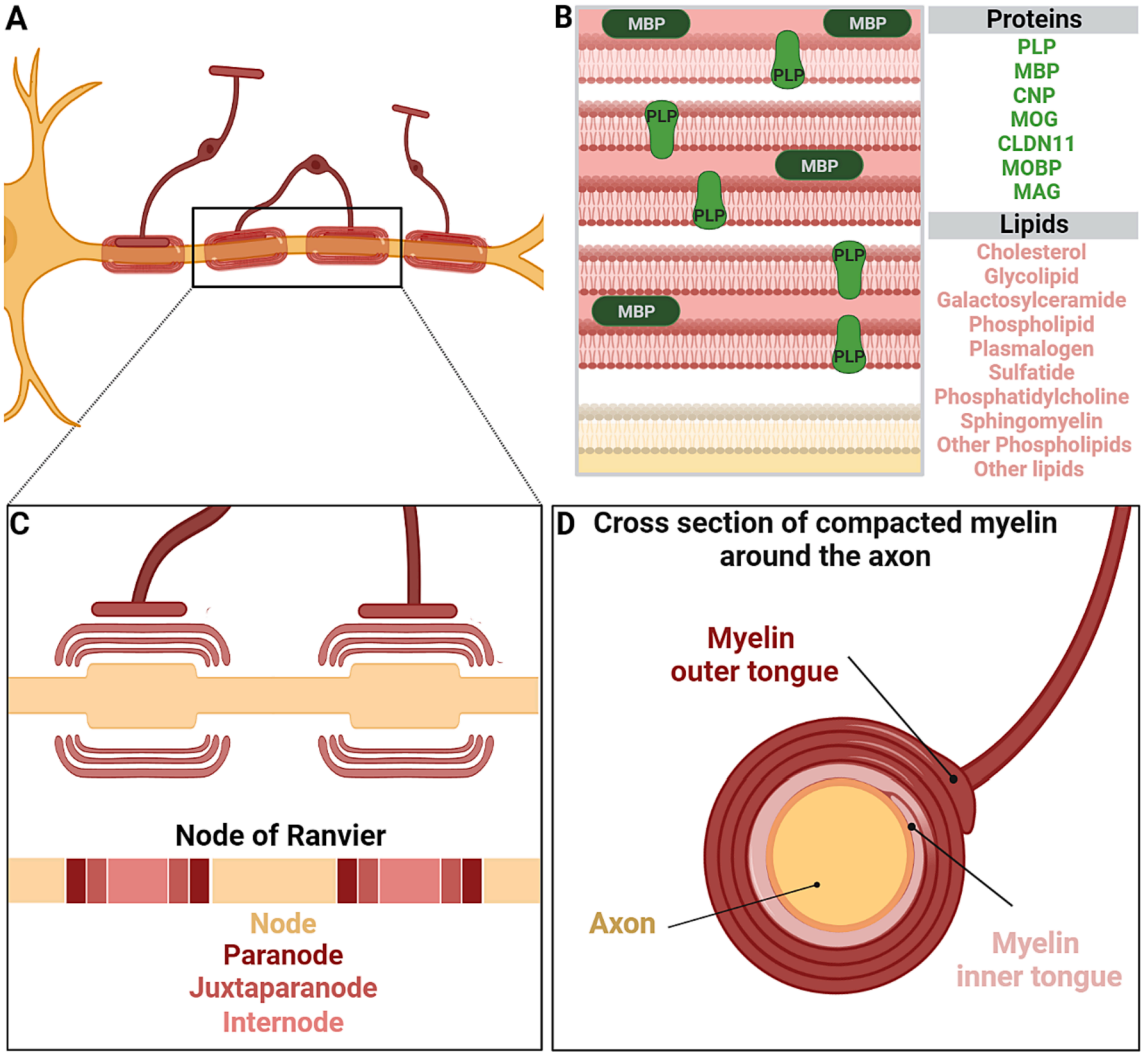

Fig. 1. Myelin sheath components and formation. In **A**, myelinated axon of the CNS is shown with oligodendrocytes extending their plasma membrane to create the myelin segments. In **B**, myelin components primarily consist of lipids (cholesterol, phospholipids, and glycolipids families) and protein, including the most abundant proteolipid protein (PLP), myelin binding protein (MBP), cyclic nucleotide phosphodiesterase (CNP), myelin oligodendrocyte glycoprotein (MOG), claudin 11 (CLDN11), myelin-associated oligodendrocyte basic protein (MOBP), and myelin associated glycoprotein (MAG). In **C**, magnified image highlights a section of a myelin sheath segment across the neuronal axon with the features of Node of Ranvier. In **D**, a cross section of an axon is graphically presented, illustrating a myelinating oligodendrocyte and the sheath around the axon.

can be altered depending on environmental cues and individual experience [76–78].

Being a complex process, myelination can be misconducted, either due to deficits in developmental myelination, or demyelination, resulting in deficient neuronal properties [15] and altered structural connectivity [79]. These deficits may result in behavioural impairments [80], defective cognition [81,82] and developmental delay [83], as in the case of several NDDs. Magnetic resonance imaging (MRI) studies in healthy children show a tight relation between early WM properties, correlating with the first wave of myelination, and the development and mastering of motor control, visual perception, and language abilities [71,72,84,85]. The difference in myelin development between children above and below average in developmental assays was consistent even when socioeconomic status and birth weight were considered [72]. In adults, WM changes are apparent following visuo-motor learning tasks [32] and new language acquisition [86]. These findings suggest that myelin plays a key role in cognitive development and skill acquisition.

3. Myelination impairments in neurodevelopmental disorders

3.1. Background

NDDs are a group of conditions associated with abnormalities of the neurological system and the brain. They manifest in early developmental stages and according to the Diagnostic and Statistical Manual of Mental Disorders-5 "are characterized by developmental deficits that produce impairments of personal, social, academic or occupational functioning" [87]. Individuals with NDDs can experience difficulties in a wide range of fields, including language and speech [88], motor skills [89], behaviour [80,90], memory [81], learning [82], and other neurological functions [91]. A higher prevalence of NDDs is found in males [92–94], and global occurrences differentiate between demographics, comorbidities, parental characteristics, and stressful life experiences [95–97].

The diagnosis of NDDs requires certain developmental milestones to be reached, but by the time the diagnostic criteria are met, the window of opportunity for effective intervention might be missed [98,99]. While the phenotype in infancy is quite similar within a disorder, as individuals grow older their symptoms might change based on environmental differences [100,101]. Early diagnosis and interventions are important, as NDDs may involve developmental disability (DD), a medical condition that poses high living costs [102,103], prevalent in 17.8 % of children in the US [96]. The symptoms of DD affect almost every aspect of life, as children are more likely to need dedicated equipment and home health care, special education, prescription medication and mental, medical, and other specialty professional help [104]. The high living cost is also contributed by loss of productivity and need for supportive living accommodation in adulthood [102].

So far, epidemiologic, genetic, epigenetic, basic molecular neuroscience, and neuroimaging studies have been precious tools to deepen and better characterize the possible causes of NDDs [105,106], the biochemical and cellular pathways that may underlie similar symptoms [107] and the overall consequences of NDDs on the affected individuals and their families. Among others, genetic and environmental contaminants are associated with adverse effects on children's brain development [108,109]. During neurodevelopment, mutations occur in genes that are primarily involved in protein synthesis, epigenetic regulation, and synaptic signalling and maturation, which all converge to common pathways of neuronal migration, chromatin remodelling, mTOR pathway, and zinc finger protein transcription factors [110]. These

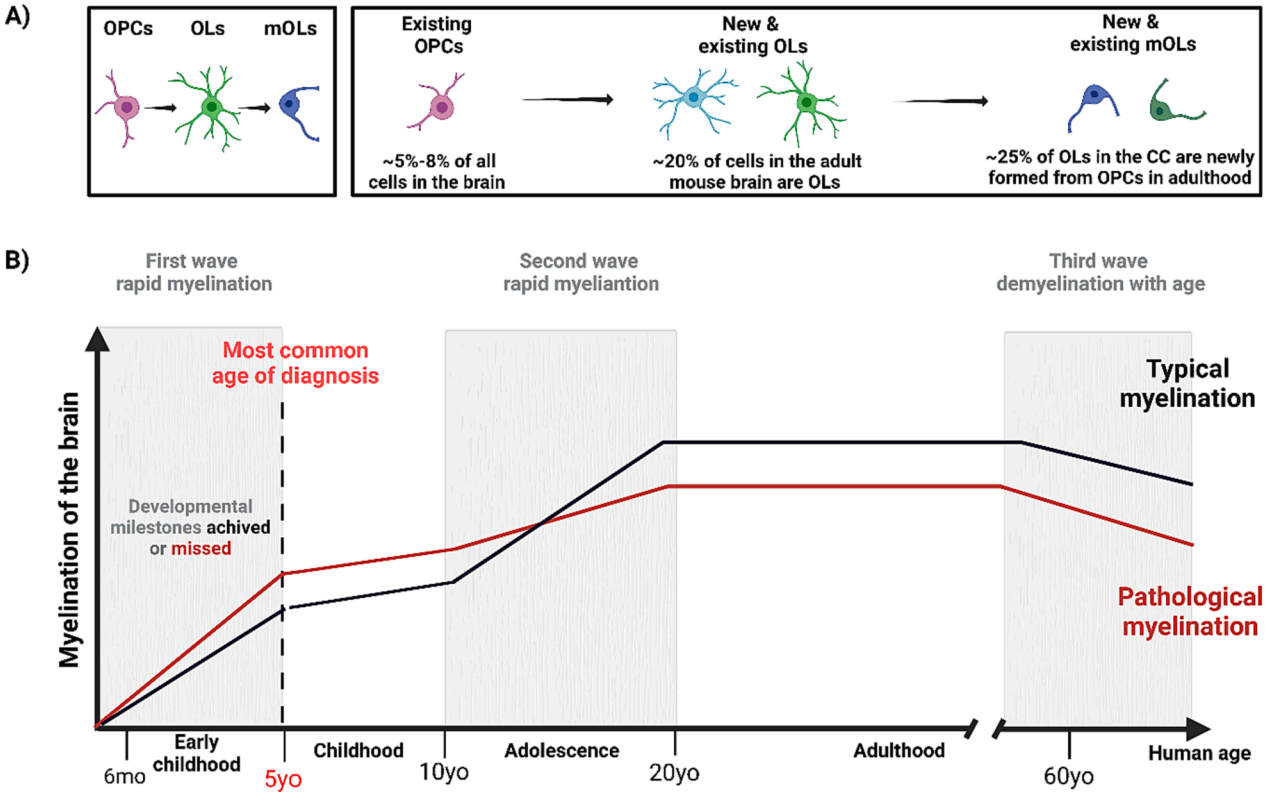

Fig. 2. Myelination of the CNS in NDDs. In **A)** the process of pre-myelinating (immature) OL formation from OPCs is presented, together with the OL maturation to myelinating mOLs during early childhood, 0–5 years of age. From childhood to ageing, myelin formation is based on existing OPCs, which generate new immature OLs that maturate to mOLs. In **B)**, a schematic graph with rates of myelination in healthy (black line) and NDDs (red line) subjects, over the human time life (expressed in years of age) is presented. Brain myelin physiological changes occur constantly along the development and can be described in distinct waves. **Abbreviations.** OPC (oligodendrocytes precursor cells), OLs (Oligodendrocytes), mOLs (mature oligodendrocytes), CC (corpus callosum), YO (years old). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pathways are also tightly connected to the onset of myelination and remyelination regulation in the CNS (e.g., BRG1 chromatin remodeller complex) [111]. The presence of heritable and *de novo* genetic components, together with individual and environmental factors, at the base of NDD's risk, makes these disorders heterogenic and complex. The multitude of processes explains the wide range of clinical signs, and the non-direct correlation genotype-phenotype, which characterize NDDs as comorbidity, and lack of specific and early pharmacological therapies.

3.2. Myelin deficits

WM abnormalities are broadly studied and characterized in both human patients and validated animal models for different disorders, including NDDs. The least invasive approach to characterize WM properties includes detection of WM tracts using the MRI-based technique termed diffusion tensor imaging (DTI) [112]. The main measurements derived from DTI are the fractional anisotropy (FA) and the mean diffusivity (MD). FA is a parameter describing the level of structural organization. Higher FA values suggest that water diffusion is highly directional, meaning an anisotropic diffusion, describing higher tissue organization (and therefore indicative of the highly myelinated fibre tracts) [113]. MD is a parameter describing the overall water diffusion without considering directionality. Higher MD values suggest that diffusion is increased, associated with tissue damage or less restricted water movement [113]. More invasive approaches that offer different resolution of indication include histology and molecular assays, for the characterizations of glia cell populations and variance in levels and distributions of myelin-related transcripts and proteins.

Brain imaging of toddlers with autism syndrome disorder (ASD) show higher FA and volume of frontal tracts at younger ages [114], and continued with age [114,115]; Infants with Fragile X syndrome (FXS) show lower FA as early as 6 months of age [116] which correlates with the cognitive impairments associated with the disorder in adults carrying the mutation [117]; In patients with Williams syndrome (WS), the overall volume of the corpus callosum is smaller [118]. As for molecular alterations: in adult mouse model of ASD, a decrease number of mOLs in the pre-frontal cortex was found, while the number of OPCs did not change [119]; In Pitt–Hopkins syndrome (ASD form), numbers of new OLs in 1 day old mice and mOLs in the adult mouse were decreased, as well as the expression of OL-specific genes [120]; In post-mortem frontal cortex tissue of WS patients, a significant decrease in mRNA level of myelination related genes was found, as well as reduced myelin thickness compared to typically developed (TD) controls [121].

These results and others are summarised in Fig. 2**B**, where pathological myelination in NDDs is presented with relation to myelination as seen in TD controls. While in the first few years of life myelination in NDDs could appear rapid and over-reaching the TD baselines, the rate of myelination is slowing down through life and the myelin content of the brain is eventually lower in NDDs compared to TD. Interestingly, the first developmental delay in NDDs can be diagnosed within the first 5 years of life [87], around the same time as the first myelination peak [63]. This finding is also replicated in mouse models, where myelin abnormalities can be studied as early as the first postnatal week, corresponding roughly with 1–3 years of age of the human brain [83].

When considering treatment, even minor improvements in performance can have immense value for the individual quality of life, and their surrounding environment. In NDDs, intervention is essential in early developmental stages, when neural plasticity [122], myelination [63], neurogenesis [123], synaptogenesis [124], and the consequent neural projection formation act at their most.

4. Pharmacological therapy for neurodevelopmental disorders

The pharmacological agents available and prescribed for patients with NDDs today are focused on symptom management rather than targeting the underlying cause. In ASD for example, atypical antipsychotic drugs are commonly prescribed for management of disruptive symptoms such as aggressiveness and irritation [125,126]. Similarly, FXS pharmacological treatments include stimulants, selective serotonin reuptake inhibitors and antipsychotics for attention deficits, anxiety, and aggressive behaviour [127]. In the case of NDDs, as the correlation between myelin impairment and cognitive deficits becomes clearer, the use of pro-myelination drugs has been increasingly considered to rescue such deficits [49,128–130].

In Nir and Barak [131], an extensive list of myelin improving drugs was published, targeting different stages of myelination, including OL differentiation and myelin synthesis. These include antihistaminic drugs (clemastine and quetiapine), antifungal agent (miconazole), anti-inflammatory drugs (corticosteroid, glatiramer acetate, nonsteroidal anti-inflammatory drugs), hormones active on peripheral and central organs (progesterone, diosgenin, thyroid hormones), agents used in neurodegenerative diseases, and other types of dementia (citicoline), anticholinergic (solifenacin and clemastine), antimuscarinic (benztropine), estrogen receptor modulators (tamoxifen, diarylpropionitrile), antipsychotic agents (quetiapine fumarate), and potassium channel blocker (4-aminopyridine). In Table 1, a list of pro-myelinating drugs has been selected from Nir and Barak [131]. The action mechanism and the function are described for each drug, together with the mechanism related to myelination.

As pro-myelination drugs are trialed and indicated mostly for multiple sclerosis (MS) [131], not much information is available on their effects in NDDs. The research that is found on the topic is very scarce and recent, like the use of clemastine [49], an antihistamine with antimuscarinic properties that has demonstrated the ability to promote OL differentiation and myelination, to rescue over-sociability in mouse model for WS [132] as well as electrophysiological deficits, hyperlocomotion and anxiety-like behavior in mouse model for ASD [132].

Recent evidence supports the contribution of astrocytes and microglial cells in the synthesis of myelin during early postnatal period [46], and the effect of drugs on these cell activities has to be taken into account while developing novel NDD therapies. This is the case for active analgesic and anti-inflammatory compounds that rescue microglial alterations and cognitive impairments in Down syndrome (DS) [133] and FXS [134] mice. Metformin, a drug commonly used for impaired glucose tolerance therapy, also rescued behavioural and cognitive phenotypes in a FXS mouse model [135] and positive effects were also observed in young children [136].

Promyelinating drugs face a significant limitation due to their poor water solubility. This challenge can be addressed through encapsulation in drug delivery systems, which serve to protect the molecule, enhance solubility, and improve bioavailability and drug efficacy. This limitation is commonly associated with the insufficient availability of the drug at the site of action. Enhancing brain availability by loading drugs into lipid-based NPs has been investigated as a promising strategy for quetiapine fumarate in rat models of schizophrenia [137,138]. The drug's effectiveness is enhanced compared to the free drug, after oral administration, highlighting the increased solubility and the improved efficacy resulting from its encapsulation. A greater transport efficiency and delivery are observed with nasal administration [139,140].

To address poor water solubility for topical treatment and skin permeation, encapsulation in lipidic and polymeric NPs can be employed. This approach enhances drug permeability through topical application while ensuring high encapsulation efficiency [141–147]. Similarly, for other drug molecules, poor water solubility and limited availability at the site of action were overcome by encapsulation in delivery systems [148–151].

Highly water-soluble drugs with a high bioavailability upon oral administration may pose a limitation, as they are rapidly and completely excreted from the body within a short time. Thus, high drug amounts need to be administered repeatedly to maintain a therapeutically viable drug concentration, potentially inducing side effects. High doses of drugs are often associated with potential toxic effects, which can be

Table 1
Mechanism of action of pro-myelinating drugs.

Drug	Action/Function	Relation to myelin
Miconazole	Inhibits the synthesis of ergosterol, a major component of fungal cell membranes.	Enhances OPC differentiation
Clobetasol	Topical glucocorticoid, binds glucocorticoid receptors and modulate expression of inflammatory genes.	Enhances OPC differentiation
Glatiramer acetate	Synthetic protein that acts as immunomodulator as a major histocompatibility complex blocker, T cell receptor antagonist, and potent approved drug for the treatment of MS.	Promotes OL synthesis and remyelination
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Inhibit the enzyme cyclooxygenase (COX) and the arachidonic acid pathway, leading to a reduction of eicosanoids produced. COX is required to convert arachidonic acid into thromboxanes, prostaglandins, and prostacyclins.	Promotes primary OL differentiation
Progesterone	Natural endogenous steroid hormone secreted by the ovaries, interacts with its specific receptors in the reproductive tract, the mammary gland, and the CNS. It negatively regulates the anterior pituitary gland in the brain to decrease follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels.	Increases OPC and mature OL density and promotes the expression of myelin basic protein (MBP) and proteolipid protein (PLP)
Diosgenin	Steroidal sapogenin, bioactive phytochemical used in the preparation of several steroidal drugs. It possesses anti-inflammatory and antioxidant properties with interest in the treatment of various types of disorders such as cancer, hypercholesterolemia, inflammation, and several types of infections.	Promotes OPC differentiation
Thyroid hormones	Triiodothyronine (T3) and thyroxine (T4) hormones are released from the thyroid gland. They play an important role in body weight regulation, energy levels, body temperature, skin, hair, nail growth, metabolism, and in the endocrine system.	Enhances OPC differentiation
Citicoline	Approved for use in stroke, head trauma and other neurological disorders, it increases phosphatidylcholine synthesis, brain acetylcholine synthesis, cell membrane repair and regeneration.	Enhances OPC proliferation
Solifenacin	Medicine used to decrease bladder activity by inhibiting contraction of the smooth muscle wall.	Enhances OL maturation
Clemastine	Selective histamine H1 receptor antagonist, blocking the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms of histamine. Clemastine fumarate suppresses neuroinflammation.	Enhances OL maturation
Benztropine	Anti-muscarinic and antihistaminic effects, selectively inhibiting dopamine transporters, histamine and muscarinic receptors. It blocks acetylcholine, and it is used to treat movement disorders like parkinsonism and dystonia.	Enhances OPC differentiation
Tamoxifen	Binds to estrogen receptors (ER), in competition with estradiol, producing both estrogenic and anti-estrogen effects. It is antagonist to eERα and blocks its signaling pathway in ERα + breast cancer cells.	Enhances OPC differentiation
Diarylpropionitrile	ERβ agonist, with anti-proliferative and pro-apoptotic effects. It also demonstrates neuroprotective effects.	Enhances OL survival and axon myelination
Quetiapine fumarate	Antipsychotic activity as combination of antagonism at dopamine receptors (D2) and serotonin receptors (5HT2A) in the brain. It is also adrenergic antagonist and potent antihistamine. Used for the treatment of schizophrenia, depression and bipolar disorder.	Enhances OL maturation
4-Aminopyridine	Blocks voltage-sensitive K^+ ion channels and increases acetylcholine levels at the synapses and neuromuscular junctions, with consequent hyperactivity, convulsions, and seizures.	Overcomes conduction failure in demyelinated nerve fibers

decreased by encapsulating the molecule, as seen for diosgenin. Encapsulation in drug delivery systems could help maintain a steady concentration of the drug at the site of action, avoiding systemic exposure to other organs [152]. Another example of a water-soluble drug is citicoline, characterized by its polar nature, that hinders its ability to cross the BBB. Loading into liposomes enhances the therapeutic effect in a rat model of cerebral post-ischemic reperfusion [153]. Interestingly, varying the phospholipid mixtures/compositions and employing different fabrication methods is a strategy to enhance encapsulation efficiency, size, plasma stability and storage. The system's flexibility has also been observed in polymeric particles where adjusting the ratio between the two polymeric monomers can improve the encapsulation and release of progesterone. This is in contrast to traditional methods, resulting in increased concentration in the blood with a much lower original dose [154].

Improving the therapeutic outcome and avoiding extrapyramidal side effects associated with chronic drug administration is another crucial aspect to consider in the development of treatment strategies for NDDs. For compounds with limited BBB passage, loading into drug delivery systems can ameliorate brain penetration and drug concentration in the brain parenchyma. Not only have nanosized systems been explored, but platforms of larger dimensions have also been investigated for topical or local administration [155–162].

5. Challenges in neurodevelopmental diseases treatment and opportunities for drug delivery

Chronic disorders, such as NDDs, are associated with long-term treatments starting from an early age, which represent a significant health care burden with social and economic implications. The major drawbacks connected with chronic treatments are the non-adherence to the therapeutic regimen, with risk of relapse and re-hospitalization, the side effect of a prolonged exposure to the drug and the compliance of patients that needs to be improved. To tackle these issues, the use of encapsulating systems with size in the nano and micro range has been evaluated as a possible strategy to prolong the release of the active compound. This approach leads to improved patient's compliance and reduced side effects, preventing relapse, hospitalization, and mortality. One of the main challenges of these systems is the presence of the BBB, a complex interface in which endothelial cells are tightly bound to protect the CNS. Crossing this barrier depends on the nature of the drug molecule and the encapsulating system [139,163–165].

5.1. Crossing the blood–brain barrier

The most selective biological barrier is between blood and the CNS parenchyma (i.e., the CNS tissue), the so-called BBB, acting as a filter of all exchanges and controls the access of cells, nutrients, and other molecules, including toxins [6]. Endothelial cells, two basement

membranes and pericytes, surrounded by end-feet of astrocytes, form the BBB.

The highly specialized and polarized endothelial cells of CNS microvessels are endowed with low endocytotic activity and bound by tight junction and adhesion molecules (occludin, claudins, and cytoplasmic scaffold proteins). Astrocyte end-feet, which are anchored through transmembrane proteins, envelop almost the entire abluminal surface of CNS microvessels, and are useful to establish the astrocyte-neuron communication. Astrocytes, the most abundant cell type in the CNS, are thus key players of the coupling between endothelial cells and neurons (the only excitable cells of the nervous tissue) in the "neurovascular unit" (Fig. 3). Pericytes exert key roles in the BBB, including the regulation of blood flow by the control of microvessel contraction/relaxation. This complex architecture provides a unique CNS interface with the blood. Perivascular macrophages and microglia represent the brain innate immune system and have a sentinel role at the BBB abluminal side.

In disease models as the BBB becomes relatively more permeable, NPs in the nano range of size (<100 nm) are significantly more efficient in crossing it. In addition, partially positive charges on the surface of the particles can facilitate the brain uptake in contrast to the negatively charged ones. However, highly positive charge is often associated with higher toxicity and rapid clearance from the blood circulation [166]. To mitigate this effect and achieve a targeted brain drug delivery system, coating with ligands is usually considered. Lately, for NPs the intranasal *(in)* administration route has been explored to facilitate the brain targeting via the olfactory nerve [167]. To bypass the BBB, the use of delivery systems is the strategy with the greatest impulse, most versatile and with promising results.

5.2. Passive and active strategies for crossing the blood–brain barrier

Different strategies are used in physiological conditions to cross the BBB: i) passive transcellular diffusion of hydrophobic molecules (<400–600 Da), hydrophilic compounds (<150 Da) and small inorganic molecules such as O_2 and CO_2 which move from the luminal to the abluminal sides of the BBB following concentration gradients, ii) active transport mediated by the interaction with specific transporters or receptors expressed at the luminal side of the endothelial cells, such as amino acids, glucose, lipoproteins, hormones, or insulin with larger dimensions [168,169].

Lipophilic molecules and drugs characterized by low polar surface and low capacity to form hydrogen bonds can also diffuse passively through the BBB. The presence of positive charges on their surfaces can increase the crossing capacity due to the interaction with the negative phospholipid heads. The active transport mainly used by nutrients (glucose, amino acids, small peptides, and organic ions) is mediated by energy-dependent transporters whose role is to remove potentially neurotoxic compounds from the CNS and limit the brain access of many drugs [170,171]. Mainly endocytosis and transcytosis, mediated by receptor binding or by absorption, drive the BBB crossing of large molecules. Macromolecular ligands, such as transferrin, lactoferrin, apolipoprotein E, leptin, or insulin, bind specific receptors on the endothelial cell membranes and form vesicles that are internalized in the cell and transported to the opposite side, where the ligands are released. In the case of albumin and other plasma proteins, the transport is mediated by protein absorption on the endothelial cell membranes, which internalize the protein and transport it from the blood to the brain parenchyma [170,171].

Approximately 98 % of small and large molecules (>1 kDa), proteins and peptides do not cross the BBB. This represents an important obstacle in the development of new therapeutics or diagnostic agents for the cure

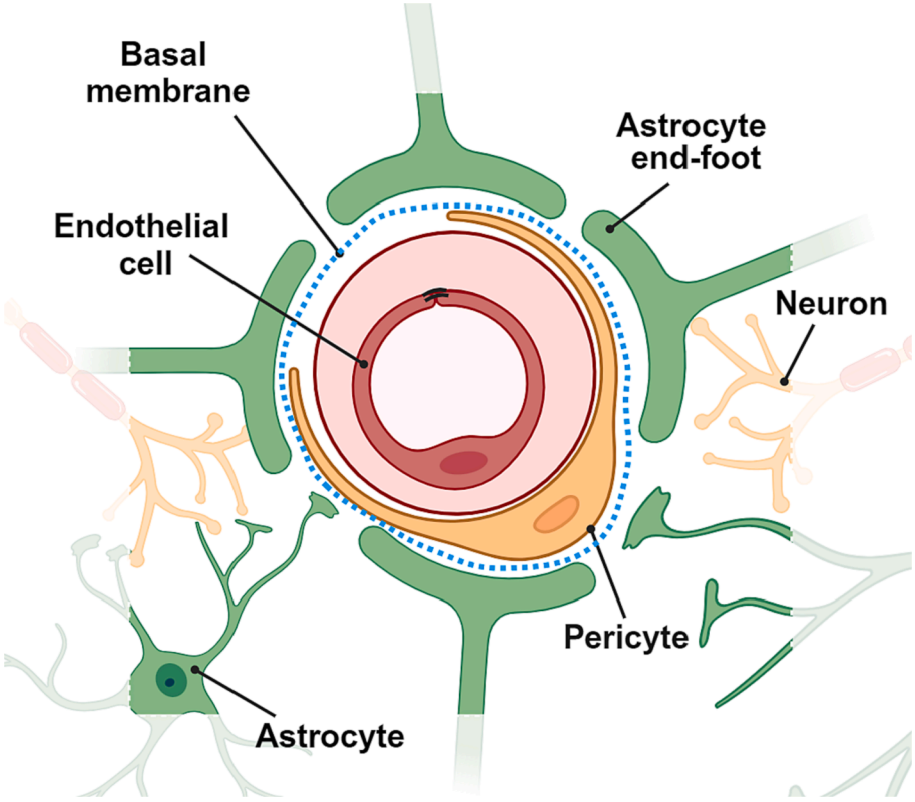

Fig. 3. The neurovascular unit of the brain. The integrity of the BBB is maintained by the presence of the neurovascular unit, which serves as the minimal functional unit of the brain. It is composed of vascular endothelial cells sealed by tight junctions and surrounded by pericytes and astrocytic end-feet. Astrocytes communicate with neuronal and glial cells. Additionally, this structure plays a pivotal role in regulating the supply of cerebral blood flow to the brain.

of brain disorders [172]. Several approaches have been attempted to deliver active compounds to the brain: from intracerebral injection to chemical modifications to increase penetration across the BBB, or binding drugs to molecules that traverse the BBB with the mediation of transporters/receptors on the cell membrane. Alternative strategies to achieve the penetration of drugs into the brain parenchyma are based on non-conventional routes of administration, such as the *in* or intraocular, which bypass the BBB, or even the induction of a transient BBB damage to alter its permeability [172–174].

With the progress of nanomedicine, there has been raising interest in systems of nanometric size as potential tools for drug transport across the BBB. NPs could load drugs that normally do not cross the BBB, and bind specific molecules or peptides on the surface to increase the passage into the brain and the targeting to a specific brain region. Several parameters drive the BBB crossing of NPs such as physicochemical properties, the interaction with circulating proteins, which depends mainly on the route of administration and is influenced by the particles size, and the presence of surface functionalization to overcome the deposit in liver and spleen and improve the brain targeting [175]. The approach of encapsulating drugs in NPs made of various lipids or polymers has been adopted also for drugs with a secondary pro-myelination effect and some of them are discussed herein.

6. Encapsulating systems for pro-myelinating drugs

The drug encapsulation strategies developed so far can be broadly classified into three main categories based on the size of the delivery vector (Fig. 4**B**). i) At the nanoscale (1–1000 nm), the primary focus of the formulation is to develop drug-loaded vectors with tissue specificity, minimise drug loss (through metabolism) and overcome drug solubility issues. Formulations in this size range are mostly suitable for systemic, *iv* and, in some cases, non-invasive administrations like nasal or topical. Because of these routes of administration and size of the particles, these formulations are rapidly excreted by the body (within a few days post injection) and are not suitable for longer release. ii) The delivery system in the microscopic range (1–500 μm) has the primary objective to obtain a steady drug concentration over a long period of time (typically more than a week), overcoming drug solubility issues and simultaneously minimizing the dosage frequency. Microscopic particles are designed for localized administrations, such as *intramuscular (im) and intraperitoneal (ip)*. The larger size of these particles results in a sustained drug release for several weeks. iii) The macro scale drug delivery systems include oral tablets or implantable systems. These systems are developed similarly to the micro sized systems, but for surgical implantation or *per os (po)* route. While *po* is the preferred method of administration, the high drug metabolism and poor bioavailability remain the main challenges. Within this category, there are also thermo-responsive injectable systems, which work as a macro drug release system but can be administered without surgical intervention. In recent years, systems where nano sized particles are combined with larger micro/macro systems have been progressively developed to synergistically improve the efficacy of the system.

In the context of neurological disorders, the challenge of having an active molecule reach the brain by traversing the BBB still persists. It is envisioned that *iv* injections of NPs loaded with potent drugs can enhance the bioavailability of molecules by crossing the BBB. These NPs are specifically designed to traverse the BBB through the active transport mechanism by incorporating binding ligands. In contrast, microparticles administered *im* or *ip* will continue to rely on the intrinsic drug BBB crossing propensity through passive transportation to reach the brain. These formulations are specifically designed to tackle the issue of repeated dosage and maintain a steady drug concentration. In fact, these formulations are restricted to drug entities with a good BBB crossing capacity. It is also possible to bypass the need to cross the BBB through *in*

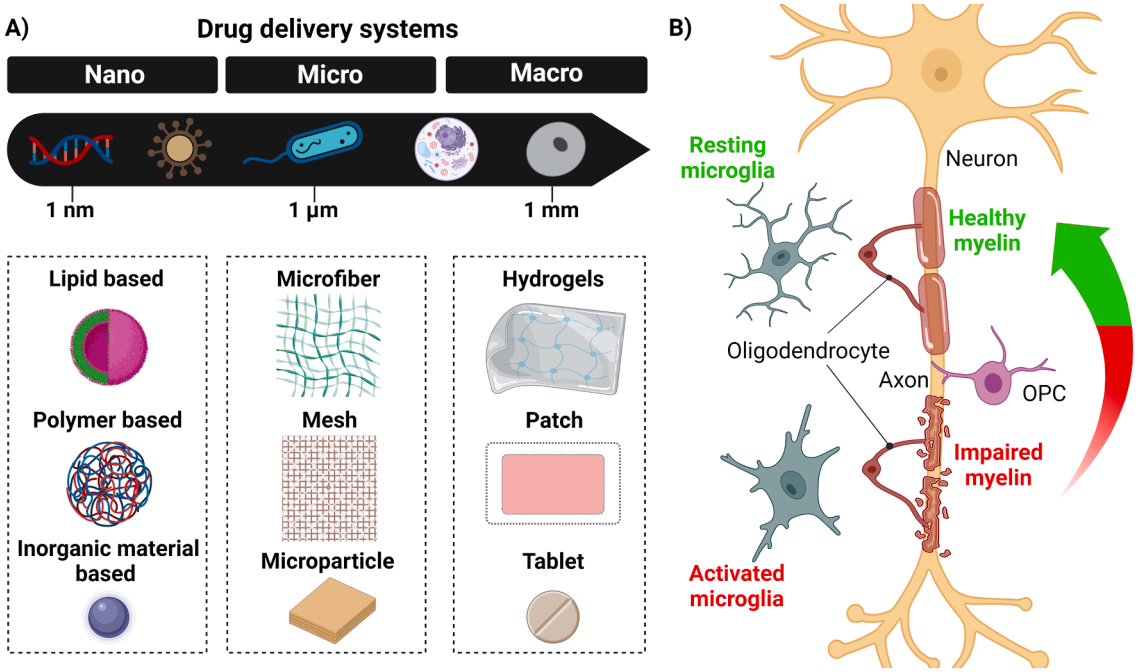

Fig. 4. Delivery systems of pharmacological active principles used in the treatment of myelin deficits. In **A)**, delivery systems of active molecules for myelinating deficits are sketched. The systems are divided into three major categories, based on their size in the nano, micro, and macro scales. The top bar reports characteristic sizes of biological systems, such as the DNA bases at 1 nm, a virus around 100 nm, a bacterial cell in the range of 1 μm, a generic animal cell in the range of 100–500 μm and a frog egg around 1 mm. The smallest delivery systems include lipid based, polymeric based, and inorganic material-based particles. The microsystems include microfibers, meshes, and microparticles. The macroscale category includes hydrogels, patches, and tablets. In **B)**, the pathophysiologic features of the myelination deficit process are illustrated. The cell types acting in this mechanism are neurons with their axons, microglial cells (resting and activated), OLs, OPCs, myelin (healthy and impaired). Delivery systems of re-myelinating drugs may improve the recovery of myelin and the physiological neuronal signal transmission along the axons.

administration of drugs loaded into nano and microparticles. However, similar to NPs administered *iv*, NPs designed for nasal administration are also intended to pass through the nasal chamber directly to the brain. On the other hand, the requirement to cross the BBB is circumvented by drug molecules loaded into macroscopic patches and devices, as surgical intervention is necessary for their implantation in the brain. Lastly, a multiscale design approach is often considered for drug entities with poor BBB crossing capacity, where the drug is loaded within a NP and then within a microparticle as a hierarchical system. While the microparticle reduces the dosage frequency, the NP assists in crossing the BBB.

The above-described drug delivery strategies can be realized using different materials that can be grouped into three categories: lipids, polymers (synthetic/natural), and inorganic materials. The choice of the material is also guided by the cost, drug nature and the envisaged administration route. Two central parameters in drug delivery are the ratio of the mass of material to the drug mass, referred to as the "loading efficiency" that gives a direct information about the drug content, and ratio of the drug recovered in a formulation with respect to the initial drug input, referred to as the "encapsulation efficiency", which indicates the efficacy of the drug encapsulation process. Developing manufacturing methods and selecting materials that could maximize drug loading and optimize its release is an ongoing challenge.

Given the emerging importance of myelination in NDDs (Fig. 4**A**), here we review studies describing the encapsulation and delivery of drugs and small molecules that do not primarily target re-myelination. However, they have shown activity in enhancing OPC differentiation (miconazole, clobetasol, NSAIDs, diosgenin, thyroid hormones, benztropine), promoting OL maturation (solifenacin, clemastine, quetiapine fumarate), stimulating OL synthesis (glatiramer acetate), increasing OPC and mOL density, and enhancing the formation of MBP and PLP (progesterone), promoting OL survival (diarylpropionitrile), or overcoming conduction failure in demyelinated fibers (4-aminopyridine). Therefore, they represent an opportunity to address myelination deficits. Interestingly, to our knowledge, almost no studies have been described up to now investigating the myelination effect using drug delivery systems and nanomedicines. This opens further opportunities for understanding drug molecule repurposing and drawing inspiration from other studies.

The delivery systems are grouped based on their size as nanosystems and micro/macrosystems (Fig. 4**B**).

6.1. *Nanoparticles for the release of pro-myelinating drugs*

Several types of NPs are under extensive investigation for neurological applications. Among them, some have been already approved by the FDA, while others are in advanced stage of development. Lipid and polymeric NPs are mostly discussed here as encapsulating systems of pro-myelination drugs, primarily developed to improve the efficacy of the drug molecules [176].

Inorganic nanoparticles

Inorganic nanocarriers are systems that do not contain carbon in their structure. They include quantum dots and metal NPs, among others, obtained from metal oxides based on gold, iron, cerium, titanium, silicon or alumina. Magnetic NPs are the most common inorganic NPs, but other metals such as nickel and cobalt have also been employed in their fabrication [177]. In the biomedical domain, the use of inorganic NPs presents notable benefits due to their different shape, wide surface area, manageable structure, diverse surface chemistry, and distinctive optical and physical characteristics. Recent global studies have demonstrated that these inorganic NPs, along with the metal ions they release, can serve as diagnostic or therapeutic agents for specific tissues or offer treatments for various diseases without causing immediate toxicity [178]. Hence, inorganic NPs show promise for drug and gene delivery due to their unique properties. However, inorganic NPs, while possessing promising properties, are also associated with potential drawbacks, including higher intrinsic toxicity, low excretion, and tissue accumulation [179,180]. Strategies such as surface coating, altering charge density and hydrophobicity, and introducing naturally derived cell membranes are all adopted to minimize cytotoxicity [177,181]. The presence of a compact core limits the drug encapsulation process for this type of NPs, but they can be easily synthesized, coated and functionalized (covalently or noncovalently) with ligands for detection and to facilitate the passive or active passage through the BBB.

However, some studies have been conducted on the effect of inorganic NPs in the context of (re)myelination. In particular, using faceted gold nanocrystals (CNM-Au8), *in vitro* maturation of OLs and *in vivo* robust remyelination were observed [182]. Robinson et al. investigated the remyelination effect of CNM-Au8 prepared as a stable, aqueous suspension of gold nanocrystals, with a 13 nm diameter. *In vitro*, the treatment of OPCs with CNM-Au8 resulted in maturation to OLs and up-regulation of myelin differentiation markers, with enhancement of aerobic glycolysis. Similarly, in co-cultured CNS cells, the incubation with CNM-Au8 led to an increased intracellular NAD^+ pool. *In vivo*, treatment with CNM-Au8 was done in a chronic mouse model of demyelination. Oral administration showed an improvement in the motor functions of cuprizone (copper-chelating toxin that induces the apoptosis of mature OLs)-treated mice. Functional myelin generation could be the result of CNM-Au8 effect on the cellular metabolism through a novel pathway involving the coenzymes NAD^+ and NADH, which are essentials for energy generation. Similarly, polyethylene glycol (PEG) coated cerium oxide NP (CeNP) decreased inflammatory process, while promoting remyelination in a mouse model of intracerebral hemorrhage. CeNP ameliorated the WM injury and inhibited astrocytes that impede microglial phagocytosis of myelin debris, resulting in remyelination and OPC differentiation after haemorrhage [183].

In addition to traditional metallic NPs, mesoporous silica NPs have also been extensively used for different applications. Specifically, immobilizing the enzyme chondroitinase ABC I (ChABC), involved in the removal of chondroitin sulphate proteoglycans and facilitates axonal plasticity, on porous silica NPs (ChABC@PSi) to increase the stability and the efficacy of ChABC. ChABC@PSi were injected in cuprizone mouse model and demonstrated a reduction in the proteoglycan content two weeks after treatment, ameliorating the demyelinated area and astrogliosis. ChABC@PSi treatment also increased the number of newly generated OPCs, promoting remyelination [184].

Organic nanoparticles

6.1.1. *Solid-lipid nanoparticles, lipospheres and nanocapsules*

In this context, lipid NPs have emerged as a promising nanoscale system into which both sparingly water-soluble and highly water-soluble pro-myelination drugs have been loaded to achieve higher bioavailability, drug protection and controlled release. Solid lipid nanoparticles (SLNs) have been explored as systems to deliver pro-myelinating drugs, such as diosgenin, citicoline, or quetiapine, to the brain [137,138,152,185] (Table 2). Diosgenin encapsulated SLNs significantly alleviated anxiety-like and depressive behavior, improved grooming behavior and social interaction in mice [152]. Diosgenin was encapsulated in Tween 80 coated stearic acid SLNs to easily reach the brain parenchyma and tested *in vitro* on glioblastoma cell line (U87-MG) and *in vivo* in the concanavalin-A–induced sickness mouse model. Diosgenin-SLN ranged from 20 to 200 nm in size, with a negative zeta potential (-26 mV), a 56 % drug encapsulation. *In vitro*, diosgenin encapsulation decreased the intrinsic toxicity of the drug. *In vivo*, free drug or diosgenin-NP were injected *ip* (100 mg/kg) at 30 min post concanavalin A injection. Behavioural tests were performed after 2 days, showing that diosgenin-SLNs significantly alleviated anxiety-like and depressive behaviors. Compared to free diosgenin, the treatment with SLNs also improved grooming and social interaction, with no toxicity indication.

Also, SLNs efficiently loaded with citicoline (CIT-SLNs) were used in the treatment of dopaminergic cells counteracting Parkinson's disease-like degenerative pathways [185]. CIT-SLNs showed a mean diameter

of 201 nm, a zeta potential of −2.20 mV, and a drug encapsulation of 80 %. Pre-treatment of dopaminergic cells (SH-SY5Y) with CIT-SLNs, and not free citicoline, before the addition of 6-hydroxydopamine to mimic Parkinson's disease effects, increased cell viability with maintenance of nuclear and cell morphology.

SLNs have been investigated as promising delivery systems of quetiapine fumarate in rat model of schizophrenia [137,138], using different routes of administration. In situ gel administration was preferred compared to *po* and *in* routes. Drug loaded SLNs obtained by micro-emulsion technique showed a size of 120 nm, a positive charge, and a 97.6 % encapsulation efficiency. Four days post-administration of SLNs, blood drug levels were comparable to the peripheral injection case and much higher than for the *po* administration, mainly due to the lower oral availability of the drug molecules. Interestingly, this system improved hippocampal morphology changes, as documented in the rat model [137]. The *po* administration of quetiapine loaded SLNs, for 3 weeks, showed an enhanced effect over free drug with a dose-dependent profile, underlying the increased solubility and enhanced efficacy of the drug upon its encapsulation [138].

Enhancing the brain drug delivery was also the aim of loading quetiapine in lipospheres for *in* delivery. These 300 nm particles showed a release of 80% of the drug at 6 h. After *in* administration in rats, the highest plasma concentration was reached by quetiapine lipospheres (22.08 μg/mL) as compared to free quetiapine suspension (6.67 μg/mL, *in*; 10.87 μg/mL *po*). Drug loaded lipospheres showed higher transport efficiency and direct nose-to-brain delivery [140]. Indeed, the nose-to-brain route represents an interesting strategy to rapidly reach the brain parenchyma circumventing the BBB [139].

Other studies have been described with delivery systems of promyelinating agents for different medical purposes (Table 2). Among others, the aim of the studies has been improving topical treatment and skin permeation of antifungal drugs [141–145,148,186,187], effect on sperm capacitation [149], and to evaluate fabrication methods to optimize drug loading and release and reduce side effects [150,151,188,189]. The poorly water-soluble miconazole was encapsulated in lipid nanocapsules, showing enhanced drug permeability through topical application, in addition to high encapsulation efficiency (>80 %) and controlled release for >48 h [141]. Similarly, when incorporated into SLNs formulation, miconazole showed a 90 % encapsulation efficiency, 23 nm in diameter, and 2.5 times higher drug bioavailability in comparison with traditional capsules used for *po* administration [145]. Clobetasol is an example of another promyelination drug where the bioavailability is limited by poor water solubility. To develop a clobetasol based topical formulation, the drug was loaded into chitosan-modified lipid NPs. Upon such encapsulation 80-fold increase in the epidermis drug localization was observed compared to the commercial form [142,143], releasing 50 % of the drug on the third day post-administration, and passively enhancement of follicular growth [144].

However, also in this case the poor water solubility and availability at the site of action remain a pharmacological challenge. Hence, as a strategy to overcome this problem, different works suggest the use of lipid NPs [148,149], lipid nano-vesicles [150] and SLNs [151] as delivery systems of progesterone, triiodothyronine (T3) and glioma-

Table 2
Lipid-based nanosystems targeting brain disorders.

Encapsulation system	Drug	Composition	Study objectives	Tests	Ref
SLNs	Diosgenin	Tween 80 coated stearic acid	Investigate anticancer and antidepressant effects	In mice (*ip*)	[152]
	Citicoline	Gelucire® 50/13	Enhance drug delivery in Parkinson's	*In vitro*	[185]
	Quetiapine fumarate	Glycerol monostearate	Investigate brain drug delivery in schizophrenia model	In rats (*in*)	[137]
		Solid lipid and lecithin	Compare drug, free and encapsulated, in rat model of schizophrenia	In rats (*po*)	[138]
Lipospheres	Quetiapine fumarate	Lecithin, stearic acid, Pluronic F127	Evaluate fabrication, *in vitro* and *in vivo* brain drug release	In mice (*in*)	[140]
Lipid Nanocapsules	Clobetasol	PCL and sorbitan monostearate	Drug penetration and distribution in skin layers	*Ex vivo* on pig abdominal skin	[186]
	Miconazole	Oleic acid and Labrafac ® oil	Enhanced topical delivery	*Ex vivo* on abdominal rat skin	[141]
	T3	PC	Compare different fabrication methods, drug loading and *in vitro* release	*In vitro* on immortal epithelial cell line	[150]
	Clobetasol	Lecithin, taurodeoxycholate, stearic acid, and oleic acid	In *vitro* skin penetration and accumulation	*Ex vivo* on pig ear skin	[142]
			Improve skin permeation	*Ex vivo* on pig ear skin	[143]
		Stearic acid, mg oleic acid, mg lecithin, and sodium taurodeoxycholate	Evaluate the hair follicole uptake	*Ex vivo* on pig ear skin	[144]
	Progesterone	Tristearin and miglyol	NPs as progesterone skin delivery systems	Tape stripping on volunteers (M, F, 25–55y. o.)	[148]
		Stearic acid or palmitic acid in different amounts tween 80	Effect on sperm capacitation and acrosome reaction in asthenozoospermia	*In vitro* on sperm cells and acrosome	[149]
	GANT61	tLyP-1 peptide-modified reconstituted high-density lipoprotein (HDL) NP	Increase bioavailability and reduce off-target toxicity in combined cancer therapy	In mice (*iv*)	[151]
	Clobetasol	Compritol 888 ATO	Topical treatment for psoriasis	*Ex vivo* permeation study (sheep skin)	[187]
	Miconazole	Precirol ATO5, Cremophor RH40, Lecinol, and Dicetylphosphate	Antifungal activity against *Candida albicans*	In rabbits (*po*)	[145]
	Progesterone	Tween 20, Octadecyl 2,3-Dihydroxypropanoate	Synthesis and characterization of a system for potential intravaginal sustained drug release	*In vitro* on agar plates	[188]
	Quetiapine fumarate	Dynasan 114, Dynasan 118, Imwitor 900P, egg lechitin,	Evaluate SLN in terms of preparation and characterization, and oral drug bioavailability	In rats (*po*)	[189]
Liposomes	Citicoline	CH, PS, PC, PA, PE	Evaluate the effect of liposomes as carriers on rat cerebral post-ischaemic reperfusion	In rats (*im*)	[153]
	Prostacyclin	Cationic lipids, stearylamine, or 1,2-dioleoyl-3-trimethylammonium propane (DOTAP)	Evaluate the pharmacologic efficacy on mouse intrapulmonary arteries	*Ex vivo*	[192]
	Quetiapine fumarate	CH. egg phosphatidylcholine(EPC) 1:2	Compare different preparations for *in* route diffusion	*Ex vivo* on sheep mucose (*in*)	[193]

associated oncogene homolog 1 (Gli1) inhibitor GANT61. Studies have shown that in addition to its anticancer effects, GANT61 also helps block inhibitory signals for OPCs recruitment and promoting remyelination [151].

6.1.2. Liposomes

Liposomes are spherical vesicular structures with one or more bilayers of natural or synthetic amphiphilic lipids. They allow simultaneous encapsulation of hydrophilic and hydrophobic substances, providing them protection and stability against external conditions. Its effectiveness may be dependent on a variety of factors: size, electrical charge, pH of the environment, lipid composition and surface modification [190]. Liposomes have revealed significant therapeutic benefits, and FDA-approved formulations are in use as antifungals, analgesics, viral vaccines, and photodynamic therapy [191].

Due to the polar nature of the drug citicoline, which prevents the BBB crossing, the molecule has been loaded in liposomes to improve the therapeutic effect and evaluated in cerebral post-ischemic reperfusion rat model (Table 2) [153]. Liposomes were prepared with various phospholipid mixtures and different methods (multilamellar vesicles or reversed-phase evaporated vesicles). The composition that gave the best encapsulation efficiency (45.6 %) was 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine monohydrate (PC): phosphatidylserine (PS): cholesterol (CH) (7:4:7 M ratio) prepared as reversed-phase evaporated vesicles. The same mixture as multilamellar vesicles, showed double size, but comparable serum stability and storage. The smaller liposomes were chosen in view of increasing the plasma lifetime, and therefore the brain localization, compared to larger liposomes. To evaluate the therapeutic effect of citicoline loaded liposomes, one-month old rats were treated with the same amount of free or trapped citicoline before and after ischaemia, obtained with the surgical bilateral clamping of common carotid arteries. PC:PS:CH (7:4:7) phospholipid mixture showed an improvement in the survival rate of ischaemic rats of 24 % compared with free citicoline, upon *im* injection. The improved therapeutic response obtained with this phospholipid composition would be due to a passive transport of the serine component, which exerts a positive metabolic role. The treatment with PC:PS:CH (7:4:7) liposomes also induced a marked reduction (60 %) in lipoperoxidation compared with free citicoline, suggesting a greater protection against peroxidative injury during ischaemic perfusion than the free drug.

Prostacyclin loaded liposomes have been investigated on vasoconstrictor diseases, showing efficacy *in vivo*, with half of the drug concentration in the liposomes compared to the free drug [192]. The problem of insufficient oral bioavailability of the lipophilic drug quetiapine fumarate was overcome using liposomal formulation developed by thin film hydration followed by sonication method. Diffusion studies were carried on using sheep nasal mucosa proving liposomal dispersion is an advantageous system for brain drug delivery through the *in* route [193].

6.1.3. Polymeric nanoparticles

Several synthetic, natural or hybrid polymers and/or copolymers are used to produce NPs. Synthetic polymers include alkyl polycyanoacrylates (PA), aliphatic polyesters such as polylactic acid (PLA), polyglycolic acid (PGA), and its copolymer poly (lactic-co-glycolic acid) (PLGA) in various proportions, polycaprolactone (PCL), and 1,2-dipalmitoyl-*sn*-glycero-phosphoethanolamin (PE). Natural polymers include polysaccharides, gelatine, alginate, collagen, chitosan, and others. The main advantage in using polymeric nanoparticles resides in the low toxicity, susceptibility to biodegradation, presence of functional groups on the surface, stability, excellent incorporation ability (encapsulation, surface adsorption, covalently bound, or incorporated into the polymer matrix), and the possibility to influence the release rate of the therapeutic agent [56].

The FDA-approved PLA, PGA, and PLGA, as polyesters, undergo simple hydrolysis of the ester base chain in an aqueous medium in nature, resulting in the cleavage of the polymer chain, which forms biologically compatible and metabolizable by-products when implanted in the body. In the case of PLGA, the biodegradability and drug release rate can be modified by varying the content of the monomers (LA and GA). The rate of degradation can also be affected by the molecular mass of the polymer, crystallinity, size, and conditions (e.g., temperature, pH, fluid flow, mechanical stress, and the presence of enzymes) [194].

6.1.3.1. Poly lactic-co-glycolic acid nanoparticles. PLGA, an FDA-approved synthetic biocompatible thermoplastic aliphatic polyester, has been extensively studied for biomedical applications. Interestingly, PLGA NPs have been already demonstrated for the encapsulation of T3 hormone, progesterone, diosgenin, tamoxifen and GANT61 [154,195–202] (Table 3).

Interestingly, T3 hormone encapsulated in PEG-PLGA NPs have been investigated in middle cerebral artery occlusion model of ischemic stroke [195]. PLGA NPs, either glutathione-coated or non-coated, loaded with T3, resulted in particles with a size of 300 nm and slightly negative charged. Both systems were equally taken up by neuroblastoma cells, with no difference in cell fluorescence intensity. *In vivo* brain uptake was also evaluated upon intraperitoneal injection of coated or uncoated NPs at a dose similar to the T3 used for stroke. One hour post-injection, both NPs showed accumulation in the brain, with greater uptake for the coated NPs. T3-NPs were then tested in the ischemic mouse model and compared to the neuroprotective property of free T3. Pre-treatment with T3 resulted in a decrease in tissue infarction (34 %) and cerebral oedema (59 %) in comparison to vehicle treated mice. Pre-treatment with non-coated and coated NPs resulted in reduction of infarction, respectively of 51 % and 58 %, and brain oedema of respectively, 68 % and 75 %, compared to controls. While the T3 solution mainly protected the brain cortex areas from ischemic damage, the increased neuroprotective activity of NPs formulations was due to greater efficacy in protecting the ischemic core area.

Recently, progesterone PLGA formulations with different configurations and polymeric matrix compositions were developed. The ratio between the two monomers lactic acid (LA): glycolic acid (GA) of 50:50 and 75:25, the progesterone encapsulation was 67 % and 57 % after 5 h, respectively with a drug release for 14 days *in vitro* [154]. Compared to traditional use of the drug, a single *i.m.* injection showed a high concentration of progesterone in the blood, with a quarter of the original dose. The solvent used to form NPs can significantly affect the release rate of the drug due to the solubility, the diffusion rate, and the depot structure, generating sustained release system also for other hydrophobic drug systems. High encapsulation efficiency (78 %), high loading capacity (8 %), good colloidal stability, and initial explosive release in an acidic medium also characterize PLGA-encapsulated diosgenin NPs showing a significantly increased drug accumulation in tumor tissues, with anti-angiogenic and anti-proliferative potential in mouse tumor xenograft model [196].

In addition, several studies described tamoxifen being encapsulated into NPs of different PLGA composition aiming at antineoplastic application. Depending on the PLGA type, solvent used, and preparation method, drug delivery profiles vary greatly, with the choice of NPs type strongly dependent on the administration route and clinical application. PLGA (85:15) NPs with a size of ~ 300 nm and a negative charge, slowly released the drug:PLGA ratio to 1:1 and did not reach 10 % after 60 days [197]. PLGA (50:50) NPs, polylysine-coated to improve NP-cell interaction, demonstrated ~ 85 % drug encapsulation rate, with controlled release over 3 weeks [198]. *In vivo*, breast tumor size was reduced up to 42 % compared to the untreated groups [199]. PLGA (75:25) NPs encapsulated ~ 58 % of drug, with a faster release (30 h), but higher cytotoxicity compared to free drug [200].

GANT61 loaded in PLGA NPs showed a high encapsulation efficiency (97 %) and a slow release of 25 % after 72 h [201]. Similarly, GANT61 and curcumin both loaded in PLGA NPs showed a controlled release of

Table 3
PLGA-based nanosystems.

Encapsulation system	Drug	Composition	Study objectives	Tests	Ref
Polymeric NPs	T3	PLGA-PEG	Evaluate drug loaded NPs in ischemic brain stroke	In mice (jugular vein)	[195]
	Progesterone	PLGA	Study of in situ depot system for sustained drug release	In rats (*in*)	[154]
	Diosgenin	PLGA	Evaluate biodistribution, pharmacokinetics, cellular internalization, and anticancer potential	In mice (*iv*)	[196]
	Tamoxifen	PLGA	Characterize drug loaded NPs for breast cancer therapy	*In vitro* on human breast adenocarcinoma cell line	[197]
		PLGA	Characterize physico-chemical properties and *in vitro* performance in human breast adenocarcinoma cells	*In vitro* on human breast adenocarcinoma cell line	[198]
		PLGA	Study the implications on oral bioavailability, breast antitumoral effect and drug toxicity	In rats (*po*)	[199]
		PLGA	Imaging and therapy of cancer cells	*In vitro* on human breast adenocarcinoma cell line	[200]
	GANT61	PLGA	Prepare and characterize NPs in terms of solubility, stability, drug delivery and toxicity	*In vitro* on human colon cancer cell line, breast cancer cell line and mouse fibroblast-like cells	[201]
		PLGA	Evaluate breast adenocarcinoma inhibition	*In vitro* on human breast adenocarcinoma cell line	[202]

56 % and 35 % within 3 days and enhanced *in vitro* antitumor activity [202].

6.1.3.2. Natural polymeric nanoparticles. Polymeric NPs obtained with natural polymers including chitosan, hyaluronic acid and silk have also been studied to improve the efficacy of pro-myelination drugs (ibuprofen, indomethacin, clobetasol, and tamoxifen) [146,203–209] (Table 4).

Chitosan is a polysaccharide-based biopolymer extensively used for developing drug delivery vectors. The presence of an amine group on the backbone of the polymer chain acts as a handle that can be used for drug binding, mucoadhesion and tissue penetration. Instead, lecithin is a naturally occurring mixture of lipidic molecules used for the synthesis of nanoparticles. The combined system of lecithin-chitosan NPs has been extensively studied due to their transdermal passage capacity aiming to synthesize the optimum nanocarrier for efficient delivery of clobetasol. Clobetasol NPs showed high encapsulation efficiency (92.2 %) and an *in vitro* accumulation in the epidermis comparable with the conventional cream, but with 10 times lower amount of drug, which is a remarkable point for reducing the side effects [206]. *In vivo*, this formulation inhibited oedema formation and showed no morphological changes in tissue or cellular infiltration after application [207].

Tamoxifen-curcumin loaded lecithin/chitosan NPs, with high encapsulation efficiency (74 % and 92 %), inhibited free radicals' production and metastasis suppression in cancer cells [203]. The potential of hyaluronic acid-coated chitosan NPs loaded with tamoxifen was evaluated in breast cancer cell lines. The encapsulation efficiency was ~ 55 %, with a slower and controlled delivery for the coated NPs, and *in vitro* cytotoxicity was significantly greater than free drug [208]. Tamoxifen-loaded chitosan NPs showed a burst release of 35 % in the first 12 h and a controlled release up to 48 h, with a dose- and time-dependent antiproliferative drug activity. Moreover, the hemocompatibility of this system makes it a promising tool for *iv* application [209]. Silk is another proteinaceous biopolymer which is being used for the development of delivery vectors due to its biocompatibility and tunable degradability. Tamoxifen encapsulated in silk fibroin NPs showed a size of 190 nm and a confinement efficiency of 79 %. The release was high in the first 6 h followed by sustained release over 48 h. The NPs inhibited tumor progression at a lower dose than conventional oral drug treatment, which may minimize treatment-related side effects [146].

6.1.3.3. Other polymeric nanoparticles. Alternative polymers, such as PCL, a biodegradable polyester, have also been used to prepare polymeric NPs loaded with promyelinating drugs (miconazole and diosgenin) [141,147,210] (Table 4).

PCL-Pluronic F68 loaded diosgenin NPs were developed to improve the performance in brain cancer therapy. They showed an encapsulation efficiency of 80 % and a burst release (first 8 h), followed by sustained and slow release (up to 72 h). *In vitro* cytotoxicity showed greater results compared to free drug [147]. Other NPs were produced by chemically conjugated diosgenin to the polymeric support, generating a methoxy PEG as a shell, conjugated drug to PCL as a hydrophobic core, and amphiphilic block copolymers to develop a code delivery system. These spherical NPs had uniform size (<200 nm), low polydispersity index,

Table 4
Polymeric nanosystems.

Encapsulation system	Drug	Composition	Study objectives	Tests	Ref
Polymeric NPs made with natural polymers	Ibuprofen	PEG-gelatin	Evaluate sustained delivery of ibuprofen-Sodium	In rats (*iv*)	[204]
	Indomethacin	Chitosan	Investigate ocular delivery of indomethacin	In rats (*ocular*)	[205]
	Clobetasol	Lecithin-chitosan	Evaluate drug transport across pig skin	*Ex vivo* on pig ear skin	[206]
			Assess the NP anti-inflammatory efficacy and tolerability	In rats (*topical*)	[207]
	Tamoxifen	Lecithin-chitosan	Characterize NP efficiency, and *in vitro* antioxidant, anticancer and antimetastatic effects.	*In vitro* on ovarian cancer cell line	[203]
			Characterize drug loaded NP and drug delivery in breast cancer cell lines.	*In vitro* on breast cancer and TMX-resistant breast cancer cell lines	[208]
			Evaluate drug delivery and cytotoxicity	*In vitro* on human breast adenocarcinoma and kidney epithelial cell lines	[209]
		Silk fibroin	Characterize drug loaded NP and the *in vitro* efficacy against breast cancer cell lines	*In vitro* on non-cancerous epithelial cell line and cancerous human breast cancer cell lines	[146]
Polymeric NPs made with other polymers	Miconazole	PCL	Assess enhanced topical delivery	*Ex vivo* on abdominal rat skin	[141]
	Diosgenin	PCL-Pluronic	Characterize NPs synthesis and evaluate effect on brain cancer cells	*In vitro* on glioblastoma cell line	[147]
		mPEG - PCL copolymer	Optimize drug release and evaluate anticancer activity	*In vitro* on human fibroblast, breast cancer, leukemia and osteosarcoma cell lines	[210]

high encapsulation efficiency (60–85 %), and good colloidal stability. The *in vitro* release profile was influenced by the pH values of the simulated medium and showed an initial burst effect followed by a slower continuous release over a period of 10 days [210].

6.2. Macrosystems and microparticles and macrosystems for the release of pro-myelinating drugs

To improve the therapeutic outcome and avoid extrapyramidal side effects associated with chronic drug administration, molecules with a secondary pro-myelination effect like progesterone, ibuprofen, miconazole, and clobetasol, have been incorporated within drug delivery platforms of larger dimension for topical or local administration [155–162] (Table 5). As per the nanoscale drug delivery systems, these micro/macroscopic platforms help overcome intrinsic pharmacological limitations of the therapeutic agents, such as the low water solubility and maintain a steady drug concentration over a longer time. In the following section, some of these drug delivery platforms are presented and critically discussed.

6.2.1. Macrosystems

6.2.1.1. Tablets. An example of a macroscopic platform for the sustained release of citicoline is the hydroxymethyl cellulose matrix tablets. Citicoline is a highly water-soluble drug with a bioavailability of 90 % upon oral administration. However, it is completely excreted from the body within 24 h hence high drug amounts are required to be administered repeatedly in order to maintain a therapeutic viable drug concentration. *In vitro* studies demonstrated that the HMC tablets could release 92 % of drug after 10 h, extending the therapeutic window of the formulation to >12 h [211].

6.2.1.2. Hydrogels. Hydrogels are made of hydrophilic polymers entrapping large amounts of water. These are highly tunable systems where physical and chemical properties can be independently tuned and controlled release of different therapeutics molecules can be readily achieved [212]. Recently, with the aim of achieving lower systemic drug diffusion and prolonged analgesic activity upon epidural injection, ibuprofen was encapsulated within a matrix of 25 % w/v Poloxamer 407 gel [155]. The pharmacokinetics of the drug in suspension versus the drug inside the gel was studied by epidural and intrathecal drug administration in pigs followed by microdialysis based sampling from the spinal spaces. When compared to the free drug, ibuprofen loading in the gel allowed 6-fold higher drug transmission into the intrathecal space required for the analgesic activity and a reduction by almost 5 times of drug diffusion from the CNS to systemic circulation.

In another case, miconazole has been encapsulated in a composite microsponge (derived from Eudragit)/hydrogel (Carbopol), to maintain a steady concentration of the drug at the site of action, avoiding systemic exposure of other organs [156]. The microsponge-containing drug, re-encapsulated inside the Carbopol matrix, improved the topical application thereby increasing the area of action when compared to a commercial formulation. The drug clobetasol was also encapsulated inside lipid core PCL nanocapsules and then introduced inside the hydrogel matrix [157,186]. Like in the previous case, this is also an example of a composite encapsulation system where a NP is dispersed into the matrix of a hydrogel to achieve a slower drug release. The effect of this encapsulation on skin permeation and penetration was investigated *ex vivo* using abdominal porcine skin membrane and *in vivo* using a dermatitis rat model, demonstrating that the hydrogel-NP composite system has a better profile than the hydrogel system. Another example of a composite NP-hydrogel system of clobetasol includes a polymer conjugated lipid NP re-encapsulated within a gel matrix of Carbopol [158]. *In vitro* studies showed a sustained release of the drug over a period of 7 days without any burst release and the Carbopol hydrogel matrix rendered 6 months of stability to the formulation when stored at room temperature. Also, *in vivo* studies investigating the non-specific drug diffusion outside the site of action concluded that this drug encapsulation method i) improved the intracellular drug uptake and ii) reduced systemic drug diffusion much more compared to a marketed formulation.

6.2.1.3. Polymeric membranes. The clobetasol residence time at the diseased site has been enhanced using electrospun mucoadhesive oral patches [159]. In this specific application, a polymeric blend of PCL, polyvinylpyrrolidone (PVP) and Eudragit was electrospun into oral patches. Polyethylene oxide (PEO) NPs were doped into the system to enhance the mucoadhesive properties of the structure. This combination of materials produced a highly flexible, nano-fibre-forming matrix with a large surface area. In addition, incorporation of drug-loaded NPs into electrospun fibers improved the overall structural integrity of the oral patches upon hydration. Clobetasol, a potent topical corticosteroid, with promising effects in promoting OPC differentiation and remyelination, can cause systemic side effects like Cushing's syndrome, hypothalamic–pituitary–adrenal axis suppression and local side effects upon over exposure. The *ex vivo*, *in vivo* and human trials showed that the drug loaded patches were adhesive, prolonged the drug contact time and stabilised drug concentration in the oral mucosa, reduced systemic drug exposure and hence are clinically acceptable as a buccal, gingivae and tongue patch.

In another study, PLGA based implantable mesh was developed for

Table 5
Micro and macro encapsulation systems.

Encapsulation system	Drug	Composition	Study objectives	Tests	Ref
Hydrogel	Ibuprofen	Poloxamer-ibuprofen	Evaluate drug-controlled release	In pigs (*epidural*)	[155]
	Miconazole	Eudragit RS100 polymer inside Carbopol gel	Study topical delivery system for diaper dermatitis	*In vitro* antifungal study	[156]
	Clobetasol	PCL nanocapsules inside Carbomer Ultrez® 10 NF at 0.5 % (w/v)	Assess drug penetration and distribution in skin layers	*Ex vivo* on pig abdominal skin and in rats (topical route)	[157,186]
		PLA-PEG NP inside Carbopol Gel	Psoriasis-like skin inflammation evaluation	In mice (*topical*)	[158]
Polymeric membranes	Clobetasol	PCL, polyvinylpyrrolidone (PVP) and Eudragit and polyethylene oxide (PEO) particles	Study mucoadhesive and drug residence in the oral mucosa	*Ex vivo* and in minipigs (*topical*)	[159]
	Ibuprofen	Engineered ibuprofen-PLGA loaded electrospun fibrous membranes	Investigate prevention of failed back surgery syndrome	In rats (*insertion on dura madre*)	[160]
Polymeric microspheres	Clobetasol	PLGA	Develop a new topical delivery system with prolonged release, reduced drug systemic absorption and side effects	*In vitro* drug release	[161]
	Progesterone	PLGA	Investigate the effect of copolymer composition on particle morphology and drug release	*In vitro* drug release	[162]

the sustained release of ibuprofen and the efficacy was tested *in vivo* in a laminectomy rat model to investigate the applicability of the system in failed back surgery syndrome [160]. To obtain a sustained release over a period of 8 weeks and minimize the burst release of the drug from the implant, the drug was encapsulated within the PLGA mesh in two different ways. While some of the drug was encapsulated in its molecular form, other drug molecules were covalently conjugated by an ester bond to a poly(2-hydroxyethyl methacrylate) (pHEMA) backbone, and the final composition of PLGA/pHEMA/Ibuprofen was electrospun into a fibrous mesh. The histological analysis showed that after a period of 8 weeks treatment, the composite system had better anti-adhesion effects compared to the untreated or the free drug loaded PLGA implants. Furthermore, the anti-inflammatory effect, based on macrophage, neovascularization, and gene expression, was greatly improved in animals treated with PLGA/pHEMA/Ibuprofen membranes. However, the primary limitation of these sustained release macro-platforms was related to the injection or implantation route, which necessarily require highly specialized skills.

6.2.2. *Microsystems - polymeric microparticles*

Another method that has been widely adopted and commercialized to achieve sustained release of therapeutics include the encapsulation of drugs within polymeric microspheres. Although different kind of polymers have been explored for the process, PLGA derived systems have emerged as the most promising ones. The controllable biodegradation, solubility in organic solvents and biocompatibility of degradation products have made this copolymer a highly attractive system for the development of sustained release platforms.

Also, in the context of drugs like clobetasol and progesterone, PLGA based microparticles have been developed to overcome some of the intrinsic limitations of the drugs. Clobetasol propionate is a potent corticosteroid, but the off-target effects were reduced by encapsulation within PLGA microspheres. Similarly, progesterone suffers from poor water solubility leading to inconsistent bioavailability and high first pass metabolism, and so it was formulated into PLGA-based sustained release system. Recently, clobetasol encapsulated within PLGA was investigated in terms of polymer chain length effect, the nature of the polymer by varying the LA:GA ratio and the ratio of the polymer and drug to obtain microparticles with varying size, porosity and drug release profiles [161]. The morphological analysis indicated that as the ratio polymer: drug was reduced, the porosity of the particles increased, leading to a faster drug release. Alternatively, drug release studies indicated that by increasing the LA content of the polymer, the rate of drug release can be reduced [161]. Similarly, by using electrohydrodynamic atomization method the encapsulation and release of progesterone, was studied [162]. Also here, the effect of varying the ratio of LA:GA was investigated with respect to the morphology of the formulation and the drug release profile. Interestingly, microparticles, obtained with LA:GA 75:25, showed an elongated shape. Instead, predominantly spherical particles were obtained when 50:50 was used. Like the previous case, it was observed that a decreased amount of polymer and an increased surface area:volume ratio led to higher drug release rate. Here, by simply varying these parameters the authors were able to slow the drug release from 76 % to 24 % after 24 h [162].

However, one notable difference between the two works is the nature of the drug release, while in the first case, there is no burst release of the drug, in the second case the drug release is biphasic in nature. This difference primarily originates from the method used for the fabrication of the microparticles.

6.2.2.1. PLGA as a monolithic sustained release platform. The success of PLGA as a material for developing injectable drug reservoirs for the long-term release of therapeutics have inspired further preclinical studies in this direction. In the context of NDDs, where chronic life-long drug administration is required, monodispersity of the formulation plays an important role, as these depots can be easily administered with less painful, lower gauge syringes, thereby ensuring higher patient compliance and treatment adherence.

By altering the size and distribution of the pores within the matrix, as compared to spherical particles, it is possible that anisotropic particles could present longer release times. Based on this concept, a monolithic PLGA-based slow-release depot system, named microplate (μPL), has been recently developed through a top-down imprint lithographic technique using a polyvinyl alcohol (PVA) sacrificial template (Fig. 5). μPL presents a square base of 20 μm x 20 μm and varying heights (5, 10 and 20 μm).

Generally, the fabrication method involved the etching of an array of wells using direct-laser-writing to generate a silicon-master-template, followed by a transfer of the pattern on a polydimethylsiloxane (PDMS) template and then to a PVA template. The final sacrificial PVA template is filled up with different polymer blends and then dissolved in water to isolate the anisotropic microparticles.

This method allows the production of μPL with a precisely controllable geometry and surface area to ensure a highly monodispersed formulation. The other important aspect is the different drug loading possibilities of these anisotropic microparticles. The loading of therapeutics is often a challenging problem, using the commonly employed emulsion-based fabrication method that often leads to denaturation or destruction of the actual therapeutic unit especially in case of unstable biologics. Furthermore, the overall ratio of the polymer to the therapeutic is an important parameter and till date the excipient in the formulation is $>$ 50 % of the actual drug content. Commonly, the therapeutic modalities can be introduced in their bare molecular form or in the form of smaller particles thereby generating a hierarchical system. Depending on the solubility of the drug, it is possible to introduce smaller NPs which are loaded with the molecular drug modalities or to introduce them in the form of solid dispersion. In case of μPL, both molecular and particulate loading of anti-inflammatory drugs like Dexamethasone or proteins like Insulin have been successfully achieved so far [213].

Also, using a similar strategy, small interfering RNA (siRNA) molecules were successfully encapsulated in polyplexes of 200 nm and loaded into μPL. This method allows loading of the therapeutic in its active form and reduce the problem of repeated injections for the treatment of osteoarthritis [207] and makes an easy tuneable system [214]. Hence, the fabrication method provides room for loading different types of cargo in different forms that are currently under investigation.

6.2.2.2. Long-acting injectable formulation: The example of risperidone. In the context of NDDs, risperidone is also a promising drug candidate, FDA-approved and marketed in various forms for schizophrenia and other psychotic disorders treatment. In fact, one of the proposed cellular mechanism responsible for the progression of schizophrenia is related to the immune activation of the microglial cells during developmental stage, leading to hypomyelination and WM integrity disruption. As a result, there is transmission desynchronization, dysconnectivity, abnormal glial cell coverage and support of synapses [215]. The current prescribed guidelines involve lifelong oral administration of neuropsychotic drugs with a low patient compliance and non-adherence to treatment regimen.

To overcome this issue, a PLGA based long-acting depot formulation of Risperidone was FDA-approved for *i.m.,* allowing to not compromise the overall treatment efficacy, while improving patient adherence due to reduced dosing frequency [216]. However, one of the main problems associated with this long-acting formulation was the multi phasic release profile with about 3.5 % of initial release followed by a 3-week lag phase of any drug release and finally the actual release for two weeks. Hence, in the lag phase, oral supplementation was necessary to maintain a steady concentration of the drug. Focusing on this drawback of the formulation, the efficacy of other PLGA compositions (50:50) was

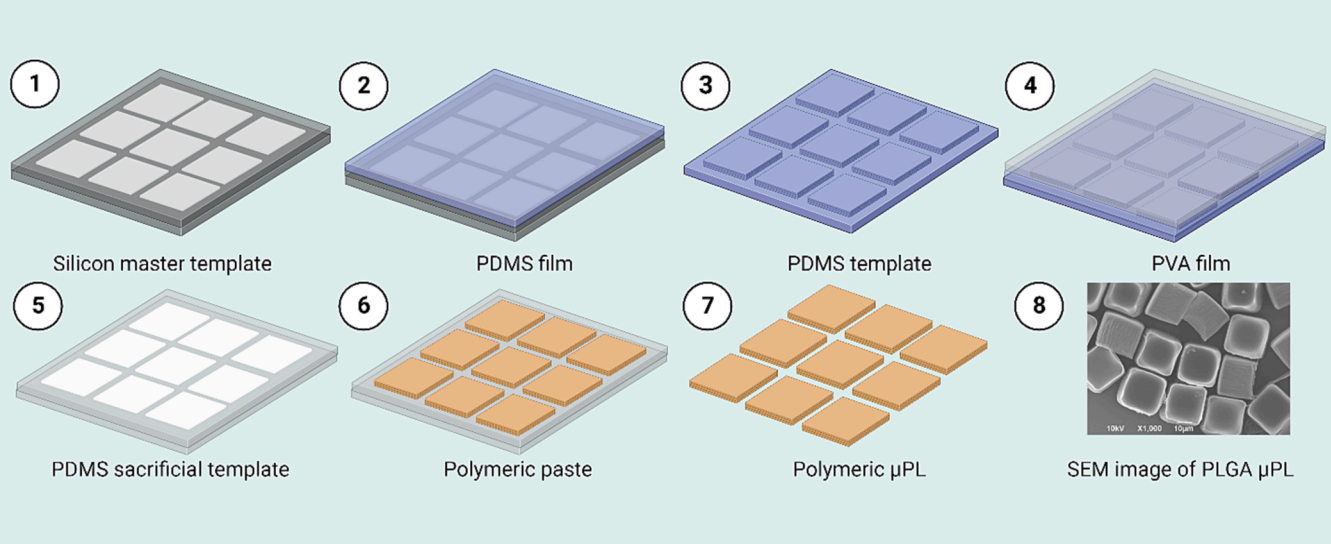

Fig. 5. Schematic representation of the top-down fabrication method for polymeric microplates (μPL). The process (from steps 1 to 8) starts from a silicon master template, which comprises a matrix of wells used to define the particles' geometry. A PDMS template is generated by replicating the original master template. This PDMS template presents a 2D matrix of pillars with the same geometry as the particles. Finally, a PVA template is produced by replicating the PDMS template. This sacrificial template presents a 2D matrix of wells, identical to the original master template, which are filled with the PLGA paste containing the drug of interest. Then, the μPL are released in an aqueous solution by progressively dissolving the sacrificial PVA template. The microscopy scanning electron microscopy image, in 8, demonstrates the defined and uniform geometry of the μPL.

studied to develop a zero-order sustained release formulation with a sustained release window of 4 weeks [217].

Thus, with the objective of developing a sustained release formulation with improved release kinetics, anisotropic square based PLGA μPL loaded with risperidone has been recently developed. Similar to the previous formulations, this system is also a bulk erosion degradation-based drug release system of PLGA [218]. Two different μPL configurations (10 μm, short and 20 μm, tall) were developed with a drug released of less than 30 % of the loaded drug within the first 5 weeks for the tall configuration.

In vivo, in a clinically relevant mouse model, empty or risperidone-loaded μPL were *ip* injected in a single administration (1.4 mg/kg), to match the total amount of free drug received in 2 weeks. The Risperidone concentration in the blood was assessed 12 weeks after a single injection of tall μPL, revealing a serum drug concentration comparable to the daily drug amount release measured *in vitro* at 100 days. Temporal object recognition test was performed after 2, 4, 8 and 12-weeks post treatment. Mice treated with a μPL single dose consistently outperformed the ones receiving daily injections or empty μPL. The system developed in this work combines the benefits of small (no lag phase) with those of larger (prolonged release) PLGA microspheres, thus extending the therapeutic window without the need of oral supplementation.

The effect of commercially available long-term release risperidone formulation (Risperdal CONSTA®) was also studied in human patients suffering from schizophrenia. The WM volume in the frontal lobe was estimated using inversion recovery MRI images from patients administered daily with an oral formulation versus a long-acting formulation. It was observed that the long-acting formulation had an improved myelination trajectory in first-episode patients and a beneficial effect on cognitive performance [219].

7. Challenges and future directions

From the clinical point of view, drug repurposing is a highly promising pathway to have a possible cure for myelination related diseases. However, a more in-depth analysis of the treatment regimen approved and prescribed, the side effects associated with chronic drug administration, the drug metabolism pathways and off target effects can help in developing more feasible therapies with improved treatment outcomes.

Among all the drugs that have shown promising promyelinating activity [131], there are some compounds that to our knowledge have not been encapsulated in delivery systems. Interestingly, only for clemastine and bazedoxifene there are ongoing clinical trials for relapsing-remitting multiple sclerosis, acute optic neuritis, and internuclear ophthalmoparesis (Table 6).

In this context, early clinical trials have established clemastine, a potent antihistaminic drug, as a promising remyelinating agent [215]. Currently it is marketed as oral tablets or in solution for the treatment of severe allergic rhinitis, seasonal rhinitis, urticaria, angioedema and common cold. Irrespective of its route of administration, similar effect was noted upon administering equal doses *po*, *iv*, or *im*. The oral bioavailability of clemastine is 40 % of the administered dose, as determined using inverse isotope dilution studies in the blood. Also, it was found to be metabolized mainly via hydrolysis of the ether linkage into multiple, pharmacologically inactive species with benzhydryl or pyrrolidine ethanol structure. This metabolism destroys the chirality of the drug molecule which is required for its antihistaminic activity. The drug undergoes elimination via the urine. There is no evidence of enterohepatic recycling, and absorption studies in rats identified the jejunum as the principal site of drug absorption. The absorption profile of clemastine was relatively slow, with C_{max} achieved between 5 and 7 h after administration while the elimination from plasma showed a $t_{1/2}$ of approximately 21 h, upon one single administration. Hence, it was concluded that repeated administration of the drug at lower concentration was a better way of maintaining a steady concentration than one administration with a higher dose.

In case of the selective ER modulators like diarylpropionitrile and bazedoxifene the generation III modulators are highly potent FDA approved drugs and have been demonstrated to have a myelin regenerative effect [220,221]. However, the approved formulation is restricted to repeated oral administration in the form of powder. Bazedoxifene has a C_{max} of approximately 2 h, with a linear increase in plasma concentrations for single doses (0.5–120 mg) and multiple daily doses (1 to 80 mg) and it showed a $t_{1/2}$ of approximately 30 h. It is

commercialised as oral film-coated tablets, also for extended and delayed release, in the treatment of vasomotor symptoms associated with menopause, acting primarily as an ER antagonist in uterine and breast tissues; it also functions in the prevention of postmenopausal osteoporosis, as it decreases bone resorption and turnover. However, the mechanism in which the drug restores the myelination process is yet to be understood clearly, one of the major side effects of long-term administration of this class of drugs can be increased uterine and endometrial growth in females and feminizing effects in men.

Considering the problems associated with the drug molecules being used in the current ongoing trials and previous studies, it is clear that further investigations could improve their repurposing for myelination in NDDs. The prescribed treatment regime, requiring multiple administrations, and long term, if not chronic treatments, clearly shows that they are inappropriate for young patients (e.g. <5 years-old). Moreover, regardless the age of the targeted population, repeated administrations are a problem which must be solved for the treatment of any chronic disease, to improve the adherence to therapy and the compliance of the patients. Drug solubility is an intrinsic property of every single drug molecule, and the drawbacks that stem from this cannot be overcome by overdosing to increase the effective drug amount. Nonetheless, overexposure to drugs could generate off target effects that are extremely important, a crucial aspect to consider in any long-term treatment. In the case of clemastine, drug encapsulation can help overcoming the specific problem of drug metabolism and maintain a steady drug concentration. Regarding selective ER modulators, like diarlypropionitrile and bazedoxifene, drug encapsulation could help reduce the administration frequency and avoid unwanted side effects. Future prospective and additional studies could consider the encapsulation of these compounds in polymeric systems, giving the high amount, side effects and the

Table 6
Ongoing clinical trials with promyelinating drugs.

Drug	Function	Clinical trial	Patients recruited	Study summary	Clinical trial protocol
Clemastine	Histamine H1 antagonist with anticholinergic properties and sedative side effects. Related to myelin deficits, it stimulates OPC differentiation and myelin formation	NCT02521311 -recruiting	All sexes, 18-55y.o. (Adult)	Assessment of Clemastine Fumarate as a Remyelinating Agent in Acute Optic Neuritis (ReCOVER) - The study aim is assessing clemastine as a remyelinating agent in patients with acute optic neuritis and tolerability of clemastine, originally approved as first-generation antihistamine.	12 mg (4 mg 3x/day) for 7 days followed by 8 mg (4 mg 2x/day) until 3 months.
		NCT05338450 - recruiting	All sexes, 18-70y.o. (Adult, Older Adult)	Clemastine Fumarate as Remyelinating Treatment in Internuclear Ophthalmoparesis and Multiple Sclerosis (RESTORE) - The study aims to assessing the long-term efficacy of clemastine fumarate in improving dysconjugacy of eye movements in patients with internuclear ophthalmoparesis and multiple sclerosis and whether a response to a single dose of fampridine can predict the effects of clemastine treatment.	4 mg twice daily (8 mg/day) orally for 6 months (180 days)
		NCT05131828 - recruiting	All sexes, 25-50y.o. (Adult)	CCMR Two: A Phase IIa, Randomised, Double-blind, Placebo-controlled Trial of the Ability of the Combination of Metformin and Clemastine to Promote Remyelination in People With Relapsing-remitting Multiple Sclerosis Already on Disease-modifying Therapy. The study goal is to establish whether the combination of metformin and clemastine can promote remyelination in people with relapsing MS, which have a greater proportion of nerves healthy enough to allow remyelination to take place.	Combination of metformin hydrochloride 500 mg prolonged release tablet for oral administration and clemastine hydrogen fumarate 1.34 mg tablet for oral administration.
		NCT05359653 - not yet recruiting	All sexes, 18-55y.o. (Adult)	Assessing Changes in Multi-parametric MRI in MS Patients Taking Clemastine Fumarate as a Myelin Repair Therapy (ReVIVE) - The study goal is to examine clemastine fumarate's protective and reparative effects as a remyelinating therapy and assessing its effect on MRI metrics of chronic lesions found in patients with a confirmed diagnosis of relapsing-remitting MS.	8 mg tablet
Diarylpropionitrile	Synthetic, nonsteroidal, and highly selective agonist of ERβ. Related to myelin, it promotes primary OL progenitor cell survival, proliferation, and differentiation *in vitro*	NCT04002934 - recruiting	Females, 40-65y.o. (Adult, Older Adult)	Bazedoxifene Acetate as a Remyelinating Agent in Multiple Sclerosis (ReWRAP) - The goal of this study is to assess the efficacy of bazedoxifene as remyelinating agent in patients with relapsing-remitting MS, using electrophysiologic techniques and MRI to quantify the effect of treatment in 50 women over the course of 6 months.	40 mg Bazedoxifene orally in 2 × 20mg blinded capsules

repeated administrations that the clinical trial protocol prescribes in adult subjects.

Developing strategies that specifically target OLs will greatly improve the potency of future potential treatments. Incorporating cell-penetrating peptides or receptor-mediated endocytosis enhancers in delivery systems may facilitate precise drug transport to the intended cellular targets. Challenges lie in addressing the BBB's selective permeability and minimizing immunogenic responses to the delivery vehicles. Future experiments might involve investigating the use of biomimetic carriers, such as extracellular vesicles or exosomes, which mimic natural biological processes. Additionally, exploring innovative non-invasive techniques like focused ultrasound for localized drug delivery to specific brain regions could revolutionize treatment approaches.

On the basic and clinical research aspects, a deeper understanding of the BBB's role in NDDs and the development of personalized medicine approaches tailored to individual patient profiles will be pivotal for advancing the field.

8. Conclusions

In this review, we discussed the different WM abnormalities that can be observed in NDDs. These myelin deficits and alterations have been found to correlate with the cognitive impairment indicative of NDDs and can be observed at very early postnatal stages of both animal models and humans with disorders such as ASD, WS and FXS. These early indications of abnormal myelin development in NDDs correlate with the first myelination wave [63].

The lack of therapeutic treatments for NDDs represents a significant health care burden, with limitations associated with a possible chronic cure. Promising advantages to tackle these issues are provided by encapsulating pro-myelinating drugs in nano/micro systems, as a strategy to extend the release of the drug over time, to reduce the administration times, and side effects and finally to increase the therapy adherence and the compliance of patients. Further investigations will consider the contribution of glial cells in the process of myelin synthesis, in the development of novel drug loaded systems in NDD therapies.

CRediT authorship contribution statement

May Rokach: Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Validation. **Corinne Portioli:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Validation. **Sayanti Brahmachari:** Writing – original draft, Writing – review & editing. **Bianca Martins Estevão:** Writing – original draft, Writing – review & editing. **Paolo Decuzzi:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Validation, Supervision, Project administration, Software. **Boaz Barak:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Validation, Supervision, Project administration, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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