Received: 1 April 2022 | Accepted: 9 June 2022

DOI: 10.1111/bpa.13105

REVIEW

Brain Pathology

Present and future avenues of cell-based therapy for brain injury: The enteric nervous system as a potential cell source

Sirine Hacene[1,2] | Alice Le Friec[2,3] | Franck Desmoulin[2] | Lorenne Robert[2] | Nina Colitti[2] | Juliette Fitremann[4] | Isabelle Loubinoux[2] | Carla Cirillo[2]

[1]National Veterinary School of Toulouse, University of Toulouse, Toulouse, France

[2]Toulouse NeuroImaging Center (ToNIC), Inserm, University of Toulouse—Paul Sabatier, Toulouse, France

[3]Department of Biological and Chemical Engineering—Medical Biotechnology, Aarhus University, Aarhus, Denmark

[4]Laboratoire des IMRCP, CNRS UMR 5623, University of Toulouse—Paul Sabatier, Toulouse, France

Correspondence
Carla Cirillo, UMR 1214 -Inserm/UPS-ToNIC Toulouse NeuroImaging Center, CHU Purpan—Pavillon Baudot, Place du Docteur Baylac, 31024 Toulouse—Cedex 3, France.
Email: carla.cirillo@inserm.fr

Funding information
Agence Nationale de la Recherche, Grant/Award Number: ANR-15-CE07-0007-01

Abstract

Cell therapy is a promising strategy in the field of regenerative medicine; however, several concerns limit the effective clinical use, namely a valid cell source. The gastrointestinal tract, which contains a highly organized network of nerves called the enteric nervous system (ENS), is a valuable reservoir of nerve cells. Together with neurons and neuronal precursor cells, it contains glial cells with a well described neurotrophic potential and a newly identified neurogenic one. Recently, enteric glia is looked at as a candidate for cell therapy in intestinal neuropathies. Here, we present the therapeutic potential of the ENS as cell source for brain repair, too. The example of stroke is introduced as a brain injury where cell therapy appears promising. This disease is the first cause of handicap in adults. The therapies developed in recent years allow a partial response to the consequences of the disease. The only prospect of recovery in the chronic phase is currently based on rehabilitation. The urgency to offer other treatments is therefore tangible. In the first part of the review, some elements of stroke pathophysiology are presented. An update on the available therapeutic strategies is provided, focusing on cell- and biomaterial-based approaches. Following, the ENS is presented with its anatomical and functional characteristics, focusing on glial cells. The properties of these cells are depicted, with particular attention to their neurotrophic and, recently identified, neurogenic properties. Finally, preliminary data on a possible therapeutic approach combining ENS-derived cells and a biomaterial are presented.

KEYWORDS
brain injury, cell therapy, enteric nervous system, glia, hydrogels

1 | INTRODUCTION

Brain injuries—including stroke, trauma, and neurodegenerative diseases—are a major public health problem. Among them, stroke is the second most common cause of death worldwide and results in severe deficits in surviving patients [1]. In 80% of cases, stroke is caused by prolonged ischemia. More than half of patients suffer from significant residual deficits, resulting in a huge economic and societal burden (6400 M€/year in Europe). Restoring blood flow, reducing cell death, and limiting secondary lesion progression are three complementary therapeutic approaches in stroke. On the other hand, stimulating tissue regeneration is an urgency, especially for patients with large debilitating lesions.

Apart from thrombolysis and thrombectomy, which can only be administered to a low number of patients in the first hours (acute phase) after an ischemic stroke, none of the available treatments currently allows complete functional recovery in patients with acquired deficits.

Regenerative medicine involves therapies that promote tissue repair and, ultimately, functional recovery

after stroke. Currently, hopes lie in cell-based therapies administered during the subacute or chronic phase to support tissue reconstruction and limit cell loss [2].

Tissue repair can occur by direct replacement of damaged cells and/or by stimulation of endogenous neurogenesis, gliogenesis, and angiogenesis using growth factors. Several cell sources are potentially available for transplantation, including neural cell lines, embryonic stem cells, mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs). The first two sources raise ethical concerns, while the capability of the other two cell types to differentiate into neurons is still very low. Stem cell therapy has thus the potential to stimulate tissue repair and regeneration, but an optimal source has not yet been found [3].

In the body, nerve cells are not only present in the brain but also in the periphery. The gastrointestinal tract is a valuable source of nerve tissue, which contains a highly organized neuronal network called the enteric nervous system (ENS) [4]. The ENS includes ganglia containing adult neurons and glial cells, as well as enteric neural precursor cells (ENPC). The latter are self-renewing and detectable in the gut even in adulthood [5]. Evidence demonstrates the presence of robust neurogenesis in the adult gut, with a remarkable rate of neuronal turnover, keeping the number of enteric neurons constant [6]. More recently, it has been demonstrated that enteric glia display a neurogenic potential [7–11]. This has a great advantage over limited neurogenesis in the brain and may have profound biological and clinical implications.

Today, therapeutic strategies based only on cells have shown their limits: high mortality and low regenerative power. Not only the cells but also their support must be considered. Indeed, the extracellular matrix is an essential component of the brain parenchyma. In view of the regulatory and safety requirements imposed by the health authorities for the production and clinical studies of Innovative Therapy Medicines, Good Manufacturing Practice and Good Laboratory Practice grade, the substitute products for the cerebral extracellular matrix are often synthetic hydrogels. These biocompatible and biodegradable compounds have been applied for nerve tissue repair and holds promises.

This review assesses the current limits in cell-based therapy for brain injuries, focusing on stroke. Trying to help overcome these limits, we also discuss the possibility to search for alternative cell sources in our body, namely the ENS, which contains neurogenic glia.

2 | STROKE

2.1 | Epidemiology, etiology, and pathophysiology

The term stroke classically covers two distinct types: ischemic, including cerebral infarctions, are the majority and represent nearly 85%; and hemorrhagic ones. Stroke is the second leading cause of death in the world, the first cause of handicap in adults and 25% of patients have severe sequelae beyond 1 year [1]. Ischemic stroke is the interruption of cerebral blood flow following cerebral arterial occlusion by an atheromatous plaque in the majority of patients (43%–80% of ischemic strokes), or by an embolus [12]. The main causes are arterial atherosclerosis, embologenic heart disease, and vasculopathy. Intracranial atherosclerotic disease (ICAD) has the highest risk of recurrent stroke compared with other stroke etiologies. Stroke patients presenting ICAD often have coexisting systemic atherosclerosis [12]. A hospital-based prospective study in patients with ICAD, reported atherosclerotic plaques in the aortic arch (60.9%) and in coronary arteries (76.9%) [13]. Thrombi can thus form in different arterial beds after the erosion of the envelope forming the atherosclerotic plaque, by inflammatory or ulcerative phenomena, and the exposure of the atheroma heart to the bloodstream [14]. The thrombus then occludes the atherosclerotic vessel or reaches distant regions by embolization. Ischemic strokes most frequently occur in the middle cerebral artery (MCA).

The blood–brain barrier (BBB) is established as a dynamic intermediary between the brain and the rest of the systemic circulation and helps maintain homeostasis of the brain environment. It is thus anatomically and functionally included in the neurovascular unit [15]. The latter comprises the main constituents of the central nervous system (CNS), including neurons, interneurons, glia (astrocytes, microglia, and oligodendrocytes), pericytes, endothelial cells, myocytes, and components of the extracellular matrix [15]. Neurons play a role of "sensors" for variations in the supply of nutrients and oxygen. They transcribe this information to adjacent astrocytes or interneurons [15, 16]. From there, the neurovascular unit triggers adjustments in the cerebral microcirculation by vasomodulation (vasoconstriction, vasodilation) [17]. Astrocytes ensure the maintenance of vascular tone. They also modulate brain plasticity and neuronal synaptic transmission, through excitatory or inhibitory gliotransmitters, such as γ-aminobutyric acid (GABA), glutamate, or brain-derived neurotrophic factor (BDNF) [18]. Pericytes provide physical support for endothelial cells and participate in angiogenesis [19]. Thanks to their contractile capacity, and like myocytes [15], they modulate vascular diameter in order to adjust blood flow to neuronal activity.

The neurovascular unit components are therefore on the front line during an ischemia–reperfusion phenomenon, during which the unit collapses and the BBB ruptures. Following cerebral infarction, the induced energy deficit causes the inability of neurons to maintain the balance of their transmembrane electrolyte gradient. This ionic imbalance is then at the origin of a disruption of neuronal signaling pathways [14], and of anoxic neuronal depolarization, promoting the release of excitatory amino acids (glutamate). Also affected by activation of metabotropic glutamate receptors (mGluR5), glial cells no longer provide extracellular

clearance of neurotransmitters. This event further increases the extracellular glutamate concentration. Neurons express *N*-methyl-D-aspartate (NMDA) receptors, involved in synaptic transmission mechanisms. In this pathological situation, NMDA receptors are overactivated by the excess of glutamate. This phenomenon is called excitotoxicity and corresponds to neuronal degeneration secondary to the overactivation of the receptors.

In addition, the imbalance of the ionic gradient induces the intracellular entry of calcium (Ca^{2+}), which is responsible of other deleterious processes, such as the activation of nitric oxide synthase (NOs) producing reactive oxygen species (ROS) and the initiation of the mechanisms of necrosis and apoptosis [14]. On the other hand, astrocytes present aquaporins (AQP), which are active transmembrane channels allowing the exchange of water molecules with the extracellular environment. During the imbalance of the electrolyte gradient, the entry of water into the cells causes pathological turgor, leading to cerebral edema, known as cytotoxic. Subsequently, other activated astrocytes proliferate (astrogliosis) and acquire a phenotype, either pro-inflammatory or immunomodulatory within 48–96 h post-ischemia [20]. Some then form a hermetic barrier, traditionally called "glial scar".

Among glia, oligodendrocytes participate in the phenomenon of Wallerian degeneration corresponding to the retrograde degeneration of nerve fibers. The initial amyloid load has been suggested to worsen the cognitive performances and impact recovery [21]. Along the same line, a persistent chronic inflammation would worsen deficits [21]. Finally, the disruption of the ascending aminergic and cholinergic fibers by the lesion was proposed to explain the decreased availability of serotonin, dopamine, norepinephrine, and acetylcholine and the occurrence of post-stroke depression, together with a diminished neurogenesis [22].

Furthermore, disruption of the BBB tends to make it permissive to peripheral inflammatory cells [23]. An inflammatory process is therefore initiated and is characterized by the rapid activation of the resident microglia, followed by the influx of macrophages, polynuclear neutrophils and lymphocytes of systemic origin [24]. Perilesional, macrophages and activated microglia secrete pro-inflammatory cytokines interferon gamma (INF-γ) and interleukin-1 (IL-1) and macrophages phagocyte necrotic debris [25].

2.2 | Recovery mechanisms

2.2.1 | Brain plasticity and compensation mechanisms

Many recovery mechanisms initiate after cerebral ischemia. A few are presented here (for review see [26]). These mechanisms are part of "brain plasticity", which is defined as the capacity for the brain to develop, modify or constitute alternative neural connections to those damaged. Brain plasticity can involve, for example, modification of synaptic efficiency or synaptogenesis (formation of synapses). "Vicariance" is a process that involves brain plasticity. It means the existence of nervous circuits capable of supplementing the function of deficient areas of the brain [27]. It may be the recruitment of adjacent, ipsilateral, or contralateral uninjured regions of the brain for the achievement of motor function. In contrast, there is compensation, which supposes the execution of a function by other mechanisms [28, 29]. Clinically, compensation would involve the use of the healthy thoracic limb when the other limb is helpless. Unlike the previous examples, a phenomenon of "maladaptive plasticity" can also occur [30] (Figure 1). When a deficient thoracic limb is no longer used for the benefit of the healthy limb in the long term, the controlesional (healthy) cerebral hemisphere is therefore recruited to perform the functions of the ipsilesional (injured) cerebral hemisphere. In this case, interhemispheric communications via the corpus callosum may result in increased excitability of the healthy cerebral hemisphere and inhibition of the injured hemisphere [31]. Brain activity is trophic for all brain areas; however, the acquired "non-use" of the deficient thoracic limb is likely to worsen its motor deficits.

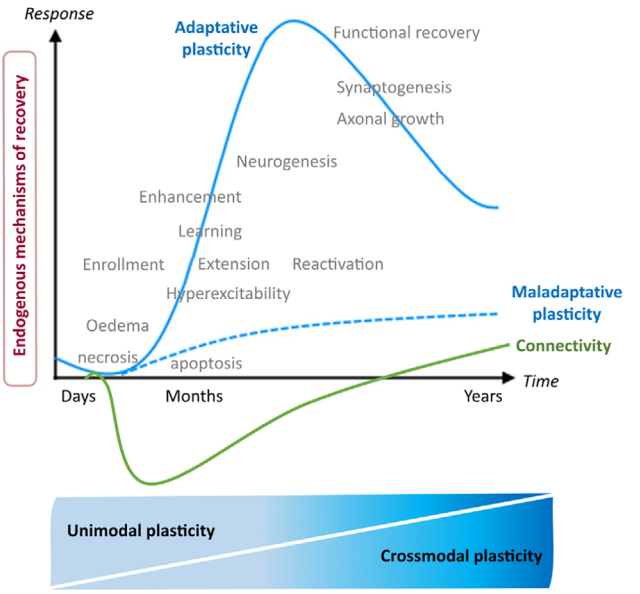

FIGURE 1 Representation of the pathophysiological, protective, and regenerative mechanisms during the functional recovery phase after brain ischemia. The two levels of plasticity (adaptive and maladaptive) are also represented. Personal realization (BioRender©), inspired by Ref. [26].

2.2.2 | Spontaneous regeneration mechanisms

Regenerative mechanisms are also involved in the recovery phase after stroke. Within the adult CNS, stem cells

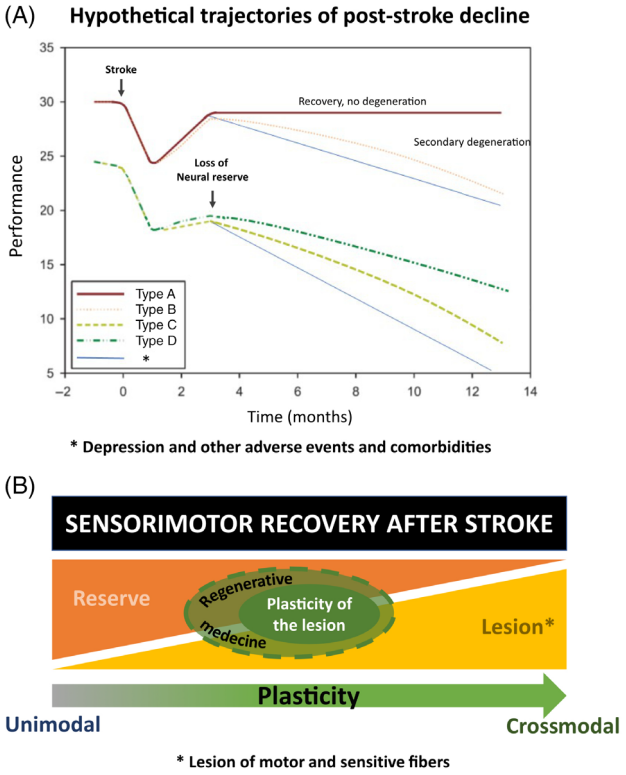

FIGURE 2 Hypothetical trajectories of post-stroke performance decline. (A) Spontaneous functional recovery in performance occurs in the subacute phase of stroke and full or partial recovery in the chronic phase is possible without further deterioration (type A). Secondary neurodegeneration can occur if vascular and inflammatory processes are present (type B). If beta-amyloid is present, basal performance may be affected, neural reserve is smaller, and recovery is affected (type C). It worsens in the event of inflammation (type D), or if depression or other comorbidities occur (*). Adapted from Ref. [21]. (B) The degree of sensorimotor recovery depends on the size of the lesion and on the destruction of sensory and motor pathways which decrease the amount of brain reserve. This concept can be extrapolated to other non-motor functions. The more the brain reserve is affected, the more brain plasticity occurs in other non-motor functions. Thus, the plasticity is unimodal (motor) at one end to crossmodal at the other end. Interestingly, neurogenesis exists which promotes plasticity of lesions. The latter can be improved by cell therapy and other regenerative strategies.

from the subgranular zone (SGZ) of the dentate gyrus of the hippocampus, the subventricular zone (SVZ) of the lateral ventricles and other circumventricular areas have unlimited proliferative capacity and the potential to differentiate into neuronal, glial, or endothelial cells [32, 33]. In a pathological context, neurogenesis is stimulated and the newly formed neurons migrate to the lesion site for cell replacement [34]. However, if a neurogenic and gliogenic potential has been demonstrated in certain regions of the brain, they remain relatively weak compared with the mechanisms of cerebral plasticity. In addition, these perilesional neurons are more likely to be involved in the modification of the microenvironment by the synthesis of trophic factors [35]. Associated with growth factors synthesized by astrocytes (BDNF, and vascular endothelial growth factor [VEGF]), these trophic factors allow the initiation of the processes of angiogenesis, axonal growth, then synaptogenesis [36]. Thus, the mechanisms of brain plasticity and tissue regeneration take place over several months (Figure 1). It has been shown that depression, inflammation, amyloid load, comorbidities and adverse events (epilepsy, pain, synkinesis, spasticity, and learned non-used) slow down or decrease recovery [26, 37] (Figure 2A). Recovery will depend on the size of the lesion and the brain and connection reserve (Figure 2B) and plasticity will be observed within one modality (motor, language) or cross-modalities.

3 | CURRENT AND FUTURE THERAPEUTIC STRATEGIES FOR BRAIN REPAIR IN STROKE

Therapeutic approaches in the field of stroke are increasingly controlled (Figure 3). As a basis for their implementation, they benefit in particular from the recommendations of the STAIR group, Stroke Therapy Academic Industry Roundtable [38] and the ADVISORY group [39], bringing together academic and industrial researchers dedicated to improving pre-clinical and clinical trials in this area. These recommendations target all stages of the development of a therapeutic strategy.

Currently available therapeutic procedures include pharmacological (thrombolytic agents and neuroprotective agents), surgical (mechanical thrombectomy, stent, angioplasty, and decompressive craniectomy) and physiotherapy. Apart from emergency management, no consensus on the treatment to be adopted has been established to date. The medical management of the patient is determined, among other things, according to the therapeutic objectives and the post-ischemic period.

Cell therapy

Therapeutic objectives

Cell therapy aims in particular to combine tissue integrity (protection, repair) and functional recovery (for review [3]). Different cell types are used and each has specific administration methods (route, dose, timing, and duration of therapy). Cell therapy is based on two main mechanisms: the release of trophic factors and/or cell replacement [40].

Cell sources and types

Several cell sources exist (Figure 4). Bone marrow (BM) is the optimal cell source, according to meta-analyses [41]. However, other sources seem more profitable, especially from a practical point of view. For example, adipose tissue is interesting [3], by its availability and its accessibility by minimally invasive methods

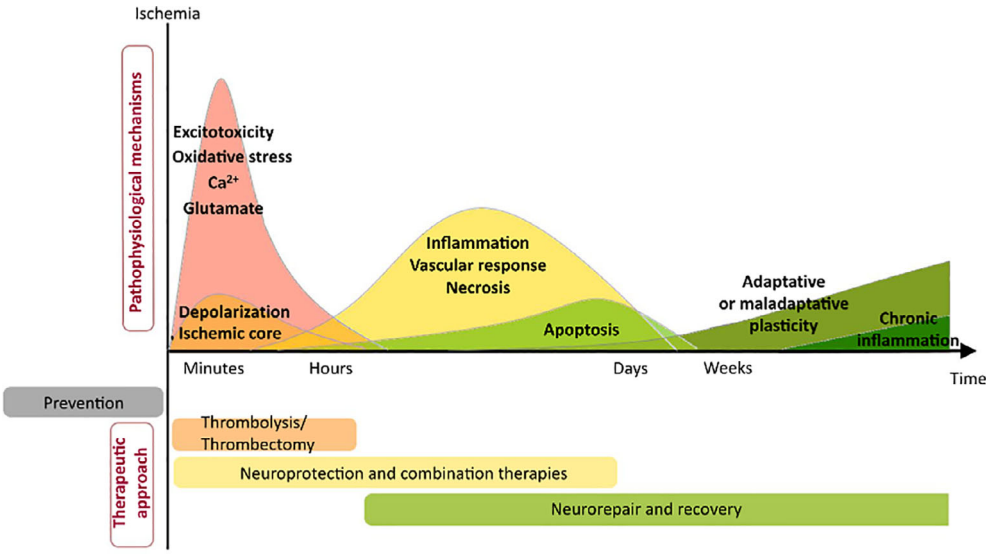

FIGURE 3 Profile of the kinetics of pathophysiological mechanisms and therapeutic approaches. Before any curative therapy, there are means of prevention (modifiable factors) that make it possible to limit the occurrence of an episode of cerebral ischemia as much as possible. Therapeutic management techniques are subsequently determined according to the post-ischemic phase. Emergency management (thrombolysis) aims to limit the worsening of lesions by promoting cerebral reperfusion. Neuroprotection techniques limit the tissue repercussions of ischemia in the subacute phase. Finally, tissue regeneration and brain plasticity are targeted in the chronic phase. Personal realization (BioRender©)

(liposuction) [42]. However, beyond practicality, the nature of the tissue influences the nature of tissue factors associated with cells, representing a limit. For example, in 2011, in a rodent model of ischemic stroke, Ikegame et al. demonstrated significant improvement (reduction in lesion volume and edema, increased concentration of proangiogenic cytokines) in individuals who received MSCs from adipose tissue, compared with those who received an BM-MSC transplant [43]. This variability is also observed for side effects, like thromboembolic risk associated with adipose tissue-MSC [44]. On the other hand, the type of cells determines the mechanism of action, and foreshadows the effects of therapy. The cell type must therefore be chosen based on the targeted therapeutic objectives, anti-inflammatory, or regenerative (acute or chronic phase).

To date, preclinical and clinical studies have tested different cell types [45], such as embryonic stem cells (ESCs), iPSCs, MSCs, adult multipotent progenitor cells (MAPCs), endothelial progenitor cells (EPCs), and mononuclear cells (MNCs) (Figure 4). However, these studies do not provide a consensus on the most efficient cell type. IPSCs correspond to somatic cells reprogrammed to return to an embryonic state and to express pluripotency genes (Oct4, Klf4, Sox2, and c-Myc) [46]. Although potentially interesting for performing autologous transplantation, iPSCs may present tumorigenic risk. In addition, a large number of stem cell sources are currently being exploited (adult neural tissue, peripheral blood, adipose tissue, BM, umbilical cord, and dental pulp). The most frequently exploited are the MSCs. They can give rise to autologous or allogeneic transplants, given their low immunogenicity [47]. They do not present a tumorigenic risk. Moreover, they tend to provide numerous trophic factors, promoting neurogenesis, synaptogenesis, and angiogenesis [48]. However, their safety remains debated so far. In addition, the persistence of stem cells at the injection site is thought to be limited (around 5%) in the days following their transplantation [49]. Thus, they are not ideal for neuronal replacement.

Finally, the last example is represented by neural progenitor cells (NPC). They participate in the endogenous neuronal regeneration of the adult brain [50]. For cell therapy, they can originate from embryonic, fetal, or adult tissue and can be easily amplified in culture [51]. In addition, they exhibit migration and pluripotency capacities [52]. However, their use remains controversial because of ethical considerations (embryonic and fetal origin) and their tumorigenic risk.

Routes and time of administration

In its early days, cell therapy was associated with the intraparenchymal route. Currently, trials are exploring the intravenous, intra-arterial, intrathecal entries [45]. Again, there is no consensus on the valid route of administration. This is to be defined according to the other aspects of the therapy (cell type, effects, and therapeutic objectives). For example, the intravenous route is easily accessible, minimally invasive, and rapid. It is nonetheless limited by a dilution effect, a risk of systemic distribution, unpredictable damage to the target organ, and possible pulmonary and renal filtration and thrombosis [48, 53, 54]. Given the nature of the pathology, the intracerebral (or intraparenchymal) stereotaxic route would

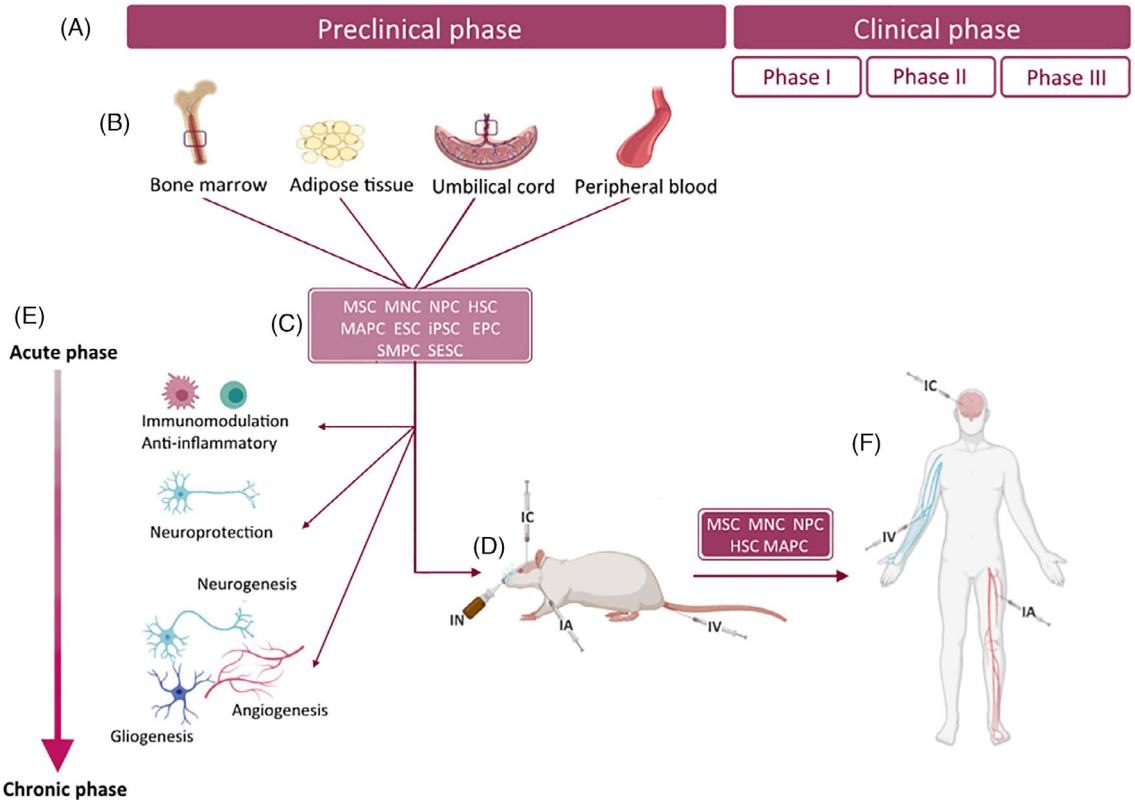

FIGURE 4 Development of cell therapy for the management of stroke in humans. (A) Development of the concept "from laboratory to clinic". The preclinical phase includes in particular the experimental steps in vitro and in vivo (animal model). It helps establish the safety and efficacy of cells used as part of a therapeutic protocol. It culminates in the development of therapy modalities for its translation to humans (clinical phases). (B) The main sources of cells: Bone marrow, adipose tissue, umbilical cord blood, peripheral blood. (C) The choice can then be made on different types of cells, having diverse properties (pluripotency, multipotency). (D) The choice of the route of administration is included in the therapeutic strategy. Different routes are accessible. (E) The pre-clinical phase allows the design of a protocol that meets the therapeutic objectives. Cell therapy can thus be carried out in the acute phase and target the mechanisms of neuroprotection and modulation of inflammation. Chronic phase therapies mainly target tissue regeneration processes (neurogenesis, gliogenesis, angiogenesis) and cerebral plasticity phenomena (synaptogenesis). (F) Finally, clinical development confirms or disproves the feasibility and effectiveness of cell therapy in humans. EPC, endothelial progenitor cell; ESC, embryonic stem cell; HSC, hematopoietic stem cell; IA, intra-arterial; IC, intracranial; IN, intranasal; iPSC, induced pluripotent stem cell; IV, intravenous; MAPC, multipotent adult progenitor cell; MNC, mononuclear cell; MSC, mesenchymal stem cell; NPC, neural progenitor cell; SESC, small embryonic-like stem cell; SMPC, smooth muscle progenitor cell. Personal realization (BioRender©), inspired by Ref. [3]

therefore be more appropriate despite their invasive nature.

In addition, the timing of cell therapy should be determined based on treatment goals. Cell therapy during the acute to sub-acute phase (until 6 months) [55] is preferably carried out in order to limit cell loss through neuroprotective and anti-inflammatory effects, or to perform cell replacement. Cell therapy during the chronic phase (>6 months) aims at functional recovery, through the release of trophic factors, for example. In general, all of the administration methods must be determined with precision in order to ensure reproducibility of the results [49].

Therapeutic mechanisms

Cell replacement. The first trials that specifically targeted cell replacement used NPCs of human or fetal porcine origin (xenotransplantation) [56, 57]. Functional improvement associated with modest cell survival and differentiation has been reported in rodent stroke models [51]. However, ethical issues, risks (zoonotic, tumorigenic) and side effects (thrombophlebitis) mitigate the results. Some trials focus more specifically on reconstruction of the neurovascular unit [3]. Among the first studies, we can find hematopoietic stem cells (HSC) or endothelial progenitor cells (EPC) exploited on a rodent model of cerebral ischemia. EPCs can integrate into the pre-existing vascular network and initiate neurogenesis [58].

Paracrine effects. Many cell types can favorably modify brain microenvironment through the release of factors. For example, dental pulp stem cells [59] exhibit immunomodulatory, neuroprotective and neurogenic effects, notably via the secretion of neurotrophic factors (nerve growth factor [NGF], neurotrophin-3, BDNF, GDNF, and VEGF) [60]. However, their safety is not yet established and their efficacy remains to be proven. Moreover,

because of the generalized damage to all the cells of the territory concerned by the ischemia (neurons, astrocytes, endothelial cells, etc.), the optimal therapy should be at most exhaustive on the regenerative effects, including neurogenesis, angiogenesis, gliogenesis, and synaptogenesis. This is particularly the case for NSC, providing neuroprotection (prevention of neuronal apoptosis), local and systemic immunomodulatory effects, stimulation of endogenous neurogenesis and angiogenesis and limiting the formation of glial scar [61]. However, their safety also remains to be validated in the clinical phase.

Translational development

The program "Stem Cell Therapeutics as an Emerging Paradigm in Stroke" (STEPS) established the main guidelines for the translational development of biotherapy [62]. These recommendations join those on animal experiments (STAIR) [63], in the matter of the precautions to be taken with regard to the transposition of a therapeutic protocol to the clinical field. The development scheme of any therapeutic strategy is conceived as follows. Launching a treatment into the market requires a set of four phases. Phase 0 corresponds to the discovery or research of a future treatment and its preclinical trials (in vitro and in vivo). The following phases (I-III) are clinical, and carried in human patients. The STEPS recommendations are then, in part, focused on the objectives of each of these clinical phases. The first phases (phases I/IIa) must show the safety and feasibility of the transplant in a context of cerebral ischemia. This includes recruiting a large panel of patients, representative of all subtypes of ischemic stroke and of varying severities [64]. Once the two parameters (safety and feasibility) have been verified, the next phase (phases IIb) can then focus on the effectiveness of the therapy. This phase involves restricting the participants as much as possible to a homogeneous group of patients (comparable functional deficit). Finally, later phases (phases II/III) should adopt the standard methodology of clinical trials (randomization, control, and blinded) to confirm the efficacy of the therapy [64] (Figure 4).

Biomaterials to help cell-based brain regeneration

Current challenges in stroke therapy are to find, on the one hand, cells capable of regenerating the adult human brain in order to restore sensorimotor functions and, on the other hand, a biomaterial serving as scaffold, suitable for neuronal differentiation. Whatever the cellular source, motor recovery is not complete in animal models, mainly because of poor engraftment [65]. Three-dimensional (3D) tissue engineering could provide solutions to improve cell survival and differentiation. This approach has been tested with the aim of replacing the 3D extracellular matrix, which is disrupted after stroke. In more specific applications corresponding to strongly anisotropic tissues, such as the spine, brain, nerves, vessels, muscles, and oriented substrates have been implemented to direct cell growth in a preferential direction [66].

In the context of stroke, strategies including biomaterials have also been investigated [66–70]. Introduction of biomaterials on the stroke site can reduce inflammation and the glial scar, promote angiogenesis and tissue reconstruction [71, 72], protect the transplanted cells and enhance their viability [73], guide the cells [74, 75], or induce a specific phenotype.

To date, a large number of soft gels are available on the market for two-dimensional (2D) and 3D cell cultures. Various biomaterials, based on polysaccharides (alginate, chitosan, hyaluronic acid), on natural proteins (collagen, fibrin), or synthetic (polylactic acid, polyacrylate or polyacrylamide polymers), can effectively support the development of cells in vitro, particularly neuronal cells, and can also provide a suitable microenvironment by releasing biological factors for cell integration, survival and differentiation [66]. Almost none, however, have all of the properties required for implantation with minimal invasive surgery and for in situ orientation. In addition, these matrices do not always give good results in terms of differentiation into neurons and in terms of 3D cell colonization of the material. Many of them are too rigid and/or chemically crosslinked. It can hinder the complete colonization of the lesion after the implantation and the elimination from the body. In addition, natural proteins and extracellular matrix extracts, which are the most represented hydrogels for 3D cell culture, do not have a well-defined composition. It varies from batch to batch and can be immunogenic.

New biomaterials such as synthetic supramolecular non-polymer hydrogels may be interesting candidates in this field. Their softness facilitates cell growth in 3D, the purity of the molecules improves the reproducibility and the fibrous microstructure tends to stimulate and guide cell extensions. They are also easily eliminated from the body. With these different aspects, they are interesting alternatives to mimic the properties of the extracellular matrix. In some conditions, the fibers can be oriented. For example, some peptides derivatives, whose structure are inspired by the typical sequences of extracellular matrix proteins (such as collagen), give oriented nanometric fibers after extrusion in vitro or in vivo. Different types of cells originating from oriented tissues (vascular, neuronal) were cultured on these fibers and resulted in the alignment of the cells along the fibrillar scaffold [76, 77]. These nanofibrillar scaffolds have provided exciting results in a model of spinal cord injury [78]. Other supramolecular hydrogels based on carbohydrates, with a fibrous microstructure, have also been used for neural stem cell culture in 3D [79].

Among existing strategies for the delivery of grafted cells to the brain, synthetic biodegradable polymer scaffolds based on polylactic-*co*-glycolic acid have been

shown to improve motor recovery in rodent models of brain injury [80].

Clinical trials

Cell-based

Numerous trials are in progress and attempt to develop the most satisfactory therapeutic strategy for ischemic stroke, from a regenerative point of view, while increasing the safety (Table 1). In 2019, Negoro et al. [81] performed the analysis of clinical trial registration data around the world (using databases: ClinicalTrials.gov and the International WHO Clinical Trials). A certain trend emerges from the analysis. In the acute phase, MSCs are used preferentially, intravenously, at high doses, in particular providing their immunomodulatory effects. In the chronic phase, various cell sources are used, especially intracranially, at lower doses. Transplants are mainly autologous (52%), to limit the risk of immune rejection. In addition, MSCs and MNCs are the most predominant cell type used for transplants, and mainly come from BM (60% of studies). Clinical trials are generally divided between phase I and phases II. For example, the project of Steinberg et al., carried out between 2011 and 2016 corresponds to a phase I/IIa, non-randomized trial, made up of a group ($n = 18$) receiving allogeneic transplantation of modified stromal cells from BM (2.5, 5, or 10×10^6), intracranially [2]. Functional assessment was performed over 2 years using neurological scales (NIHSS and mRS). The results show the feasibility and safety of the transplant, and the improvement in functional scores (except for the mRS score). In another neuropathological disease close from stroke, traumatic brain injury, a multicenter trial STEMTRA demonstrated the safety and Class I efficiency of the same mesenchymal SB623 cells. Motor status was improved ($n = 61$ patients) [82]. Motor recovery was also improved after intravenous graft of autologous MSCs and fMRI activation, used as a fMRI biomarker, was enhanced in the primary motor cortex in grafted patients ($n = 31$ patients) [83]. Finally, another trial PISCES, a phase 1, first-in-man study, investigated the safety and tolerability of human NSC treatment injected intracranially in stroke patients [84]. Together with no adverse events, the study evidenced a second endpoint: the improvement of neurological function in chronic patients (NIHSS) and/or Action Research Arm Test (ARAT) in less severe patients (PISCES-2) [85].

Some studies attain the last clinical stages. For example, MultiStem® Administration for Stroke Treatment and Enhanced Recovery Study (MASTERS-2) is a randomized, quadruple-blind, control study, started in 2018 and injected intravenously allogeneic BM-MSCs, in the case of ischemic stroke [86]. No significant improvement was seen at 90 days post-stroke.

Biomaterial-based

Biomaterials are introduced either as carriers for localized drug delivery (particles), or for cell delivery or cell guidance (scaffolds). The relevance of strategies including either particles for drug delivery or scaffolds, has been analyzed in a meta-analysis of 66 pre-clinical studies of ischemic stroke [66]. They concluded in a higher improvement of neurologic score in the presence of the biomaterial [87]. A copolymer of poly-lactic-*co*-glycolic acid (PLGA) and poly-L-lysine (PLL) has also been tested after spinal cord injury in rodents and primates [80]. The INSPIRE study was a first-in-human study demonstrating that the implantation of the scaffold within the spinal cord was safe and associated with a 6-month improvement that exceeded historical controls [88]. The bioresorbable polymer scaffold was intended to serve as an extracellular matrix, minimize expansion of areas of necrosis and cyst, support endogenous repair processes following injury and increase neural sprouting. There were no severe adverse effects related to the device, the implant or the implantation procedure. The potential benefits of such biomaterial-based strategy outweighed the risks in this patient population and support further clinical investigation in a randomized controlled trial.

Current needs and perspectives

What emerges from the literature and clinical trials is the need of cells with no immunogenicity, easy to harvest and amplify, not requiring genetic intervention, capable of migrating exclusively to the lesion site and administrable via a safe and well-tolerated route. Future studies should thus identify novel and valid sources of cells to be used for therapy, with three conditions to be met by the engrafted product: (1) (long-term) safety; (2) survival; and (3) anatomical and functional integration within the host tissue.

In humans, nerve tissue is not only present in the brain but also in the periphery. For example, the gastrointestinal tract contains a highly organized network called the ENS, which is referred to as the "second brain" [4]. Evidence demonstrates the presence of robust neurogenesis in the adult gut, with a remarkable rate of neural turnover, which maintains the number of enteric neurons constant. This is a great advantage compared with the limited neurogenesis in the CNS. It may drive profound biological and clinical implications. The second advantage is that, compared with the CNS in the brain, the ENS in the gut is easily accessible. Indeed, via routine biopsies, a small 7–10 mm^2 tissue sample can be harvested that contains a valuable number of ganglia (8/mm^2), with neurons (5/ganglion) and glia. Following, we discuss about the innovative idea that the ENS could be a valuable source of cells for regenerative strategies.

TABLE 1 Summary of the key cell-based clinical trials and their advantages/limitations. Several clinical trials have made it possible to progress the search for a valid cell source for transplantation in the context of ischemic stroke. The following table summarizes the pros and cons of these trials.

Clinical trials	Advantages	Limitations
Stem cells (SCs)	• A large number of stem cell sources: adult neural tissue, peripheral blood, adipose tissue, bone marrow, umbilical cord, dental pulp, including source as adipose tissue, available and accessible by minimally invasive methods (liposuction) • Any tumorigenic risk • Provide numerous trophic factors, promoting neurogenesis, synaptogenesis, and angiogenesis [48]' via the secretion of neurotrophic factors (nerve growth factor [NGF], neurotrophin-3, BDNF, GDNF, VEGF) [60], limiting the formation of glial scar [61]	• Controversial safety • Unproven efficacy • Limited persistence of stem cells at the injection site (around 5%) in the days following their transplantation [49] • Thromboembolic risk associated with certain factors (adipose tissue-MSC) [44].
Progenitor cells (PCs): *neural progenitor cells (NPCs) or endothelial progenitor cells (EPCs)*	• Various sources: embryonic, fetal, or adult tissue [51] • Used in cell replacement strategies, exhibiting migration, pluripotency capacities [52], and differentiation [51] • Participate in the endogenous neuronal regeneration of the adult brain [50] • Integration into the pre-existing vascular network and initiating neurogenesis (EPCs) [58]	• Ethical concerns (embryonic and fetal origin) • Zoonotic risks • Tumorigenic risk • Frequently reported side effects (thrombophlebitis) • Modest cell survival
Induced pluripotent stem cells (IPSCs)	• Pluripotency capacities, enhancing endogenous neuronal regeneration • Low immunogenicity: accessible for performing autologous or allogenic transplantation, with minimal invasive methods of sampling [47]	• High tumorigenic risk

4 | THE ENTERIC NERVOUS SYSTEM

The premises of knowledge concerning the enteric nervous system (ENS) date back to the study by Starling and Bayliss, in 1899, describing the mechanisms of innervation of the small intestine [89]. The different characteristics of the ENS will be then gradually revealed over the following decades. A new associated discipline was then created, called neurogastroenterology. As well described, the ENS shares morphological and functional features with the CNS. Studies have revealed in the ENS markers that are also expressed in the CNS [4]. In addition, the ENS and the CNS share common neurotransmitters, which enable continuous bi-directional communication through the brain–gut axis. These analogies let hypothesize that ENS-derived cells could serve as strategy for brain injury, although, so far, preclinical studies have focused on ENS repair.

Anatomy

The ENS is one of the most extensive and complex structures in the peripheral nervous system (PNS). The activity of the ENS remains independent of the CNS [4] (Figure 5). However, the ENS takes on structural and functional characteristics similar to those of the CNS: it is made up of two large groups of cells, neurons and enteric glial cells (EGC), which are grouped in interconnected ganglia (Figure 6) [4]. Together, they constitute the intrinsic neuroglial network of the gastrointestinal tract, of which the two main continuous layers are: the myenteric and the submucosal plexus [90]. The myenteric plexus is the most extensive, located between the circular and longitudinal muscle layer, and traversing the entire digestive tract, from the esophagus to the internal anal sphincter [4]. As for the submucosal plexus, it is less extensive and located between the circular muscle layer and the intestinal submucosa, mainly in the small and large intestine [4]. The myenteric and submucosal layers are distinguished by the density of neurons and EGC.

Organogenesis

During embryogenesis in vertebrates, the formation of the ENS is initiated by the invasion of the esophageal mesenchyme by enteric precursors derived from neural crest cells (NCSs), called pre-enteric neural crest cells (pENCC) [90]. Subsequently, the latter migrate rostro-caudally through the digestive tract and colonize the myenteric region. A few days later, they undergo a radial migration toward the submucosal region of the gastrointestinal tract [91]. Precursors derived from the rostro-truncal neural crest colonize the esophagus and the proximal end of the stomach [92]. Those of the vagal neural crest colonize most of the intestinal tract [92, 93]. Finally, those of the sacral neural crest colonize the distal part of the intestine [94]. At this stage, pENCCs become enteric neural crest cells (ENCC) [95]. The latter proliferate and make a final rostro-caudal migration, in order to colonize the entire digestive tract [95]. During this phase, a pool of ENCCs simultaneously initiate neuronal

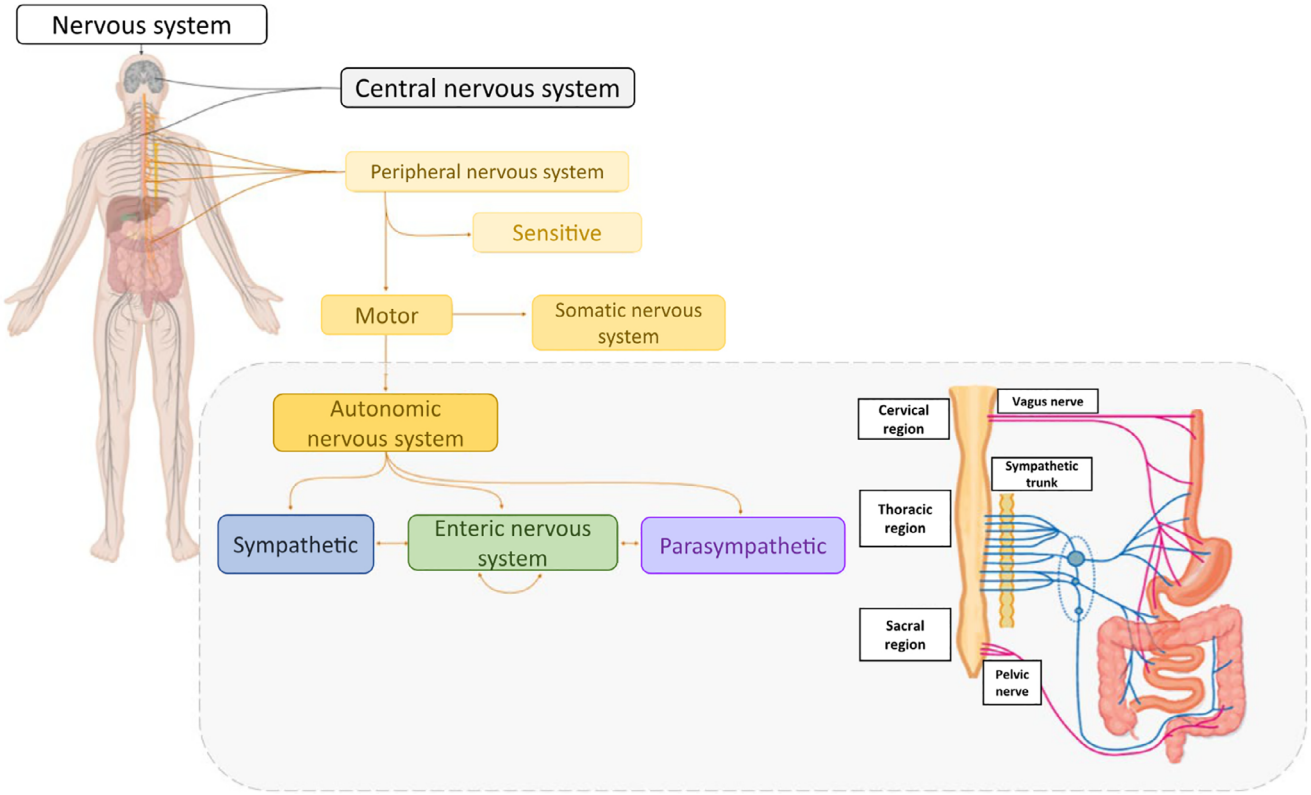

FIGURE 5 Organization of nervous systems. The enteric nervous system (ENS) is part of one of the divisions of the peripheral nervous system (PNS). More precisely, the motor subdivision of the SNP branches into the somatic (voluntary activity) or autonomic (involuntary activity) nervous system. The latter includes the sympathetic, parasympathetic and the ENS [99]. Extrinsic innervation of the digestive tract is made up of the sympathetic and parasympathetic system (via the vagus nerve and the pelvic nerve). Personal realization (BioRender©)

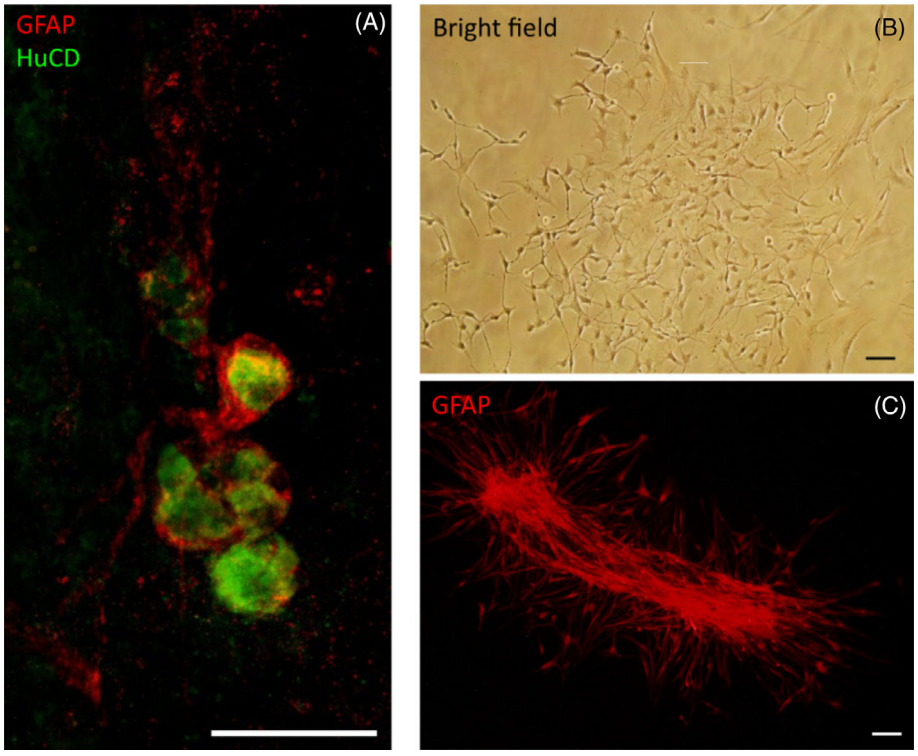

FIGURE 6 Glia in the ENS. (A) Typical ganglion in the submucosal plexus isolated from human biopsy. Note the network that EGC (GFAP, red, 1:1000, AbCam) form around neurons (HuCD, green; 1:500, Invitrogen). Images were recorded using Zeiss LSM 710 confocal microscope (Cell Imaging Facility, INFINIty, Toulouse). Max intensity image was obtained with ImageJ. (B) Bright field image of human primary EGC isolated from the submucosal plexus of colon biopsies. EGC, like brain astrocytes, have a star-like shape in culture. (C) Human primary EGC in culture stained with GFAP antibody (1:1000, AbCam) with the secondary antibody donkey anti-rabbit Alexa Fluor 594 (1:1000; Invitrogen). Scale bars: 50 μm

and glial differentiation [96]. Additionally, the contribution of Schwann cell precursors (SCPs), a neural crest-derived stem cell pool, to ENS innervation has been demonstrated [97, 98]. During the postnatal period, a subset of these cells invades the gut alongside the extrinsic nerves, adopts a neuronal fate and contributes to the ENS. This contribution has been quantified as approximately 20% of enteric neurons. However, SCPs contribution is limited to the colon, while in the small intestine they are present only in a restricted percentage of submucosal neurons [97].

The ontogeny of the ENS ends with the configuration of cells into interconnected enteric ganglia and the formation of neural circuits [90, 91, 96].

The normal development of the ENS is mediated by numerous regulatory molecules [99], including the transcription factor Sox10 and the receptor tyrosine kinase Ret [99]. During embryogenesis, the expression of *Sox10* by ENCCs is necessary for their survival and maintenance of their multipotency [100]. As for Ret, the latter is a receptor for neurotrophic factors derived from the glial cell line (GDNF) [101], essential for the chemotaxis of ENCCs along and deep in the wall of the digestive tract [101]. In addition, it is involved as a signal for proliferation and neuronal differentiation of enteric precursors, and in neuronal survival [102].

Numerous in vitro studies have demonstrated the modulation of the expression of the transcription factor *Sox10* and the Ret receptor by enteric progenitors during their differentiation. In essence, downregulating *Sox10* expression and maintaining that of Ret promotes the formation of enteric neurons (Sox10−/Ret+) and that of neural subtypes. Conversely, maintenance of Sox10 expression and downregulation of Ret expression promotes the formation of EGC (Sox10+/Ret−) [103].

Physiology, functions, and composition

The ENS is not structurally and functionally isolated. It maintains many dynamic interactions with components of other nervous systems, but also with the intestinal epithelium, microbiota, muscle, endocrine, endothelial, and immune cells (for review see [104]). The gastrointestinal tract performs many vital functions, such as digestion, absorption, secretion, and peristaltic movements. For this, the tract is under the control of a double innervation. A first intrinsic innervation is established by neurons and glial cells of the ENS. A second or extrinsic innervation is performed by neurons of the sympathetic and parasympathetic system (via the vagus and pelvic nerve) [94] (Figure 5).

Neurons in the adult mouse ENS can be distinguished in two main classes, based on their electrophysiological and morphological characteristics: AH neurons and S neurons. Morphologically, they have peculiar features: AH neurons have Dogiel type II morphology with a smooth cell body and multiple circumferentially-projecting axons; S neurons are uniaxonal (Dogiel type I morphology) and have short lamellar dendrites [105, 106]. The difference in axon and dendrite organization explains the distinct projection of AH and S neurons. Indeed, Dogiel type II neurons supply branches within enteric ganglia, circumferentially. S/Dogiel type I project orally or anally to the circular muscle, to the longitudinal muscle and to other ganglia. The classification of ENS neurons has been enriched by the identification of specific neurotransmitters and target tissue and direction: 10–15 myenteric and 4–5 submucosal neuron types have been described in guinea pig and mouse. Major circuit features have been identified: intrinsic (sensory) primary afferent neurons (IPAN), intestinofugal neurons (IFAN), ascending and descending interneurons, excitatory and inhibitory muscle motor neurons [4]. IPANs regulate the status of the intestinal lumen and the homeostasis of the intestinal wall, and together with IFAN, are afferent neurons with their cell bodies in the gut wall, without connections to the CNS [107]. Interneurons provide the interface between the neurons in different ganglia.

In humans, enteric neurons have not been systematically catalogued; so far, myenteric and submucosal neurons are identified as Dogiel type I, II, or filamentous and their morphology matches with S and AH type electrophysiological properties [108]. Quantitative data regarding enteric neuron subpopulations in human tissue are scarce and conflicting, mainly because of the limited availability of control tissue [109]. In addition to neurons, the ENS includes a population of EGC, which outnumber neurons [110]. For example, in humans, the number of EGC is approximately seven times greater than that of neurons [111]. This observation remains valid in other species of mammals [111]. Within enteric ganglia, neurons and EGC exhibit close membrane contacts [112] and form a cohesive whole in the absence of cohesive cells or collagen fibers. These characteristics thus echo those of the CNS between neurons and astrocytes.

4.1 | Enteric glial cells

4.1.1 | Uniqueness of cell type

It was in 1899 that Dogiel observed and described EGC for the first time [113]. However, it took several decades before a particular interest in these cells flourished in researchers. EGC were initially defined as Schwann cells, but the work of Gabella finally established their uniqueness in 1972 [114]. These cells are now recognized as phenotypically and functionally distinct from all other known glial cells [115].

4.1.2 | Types and locations

Numerous studies have demonstrated the plasticity and heterogeneity of EGC. Despite they come from a single pool of NCCs, the conditions of their microenvironment

determine the phenotype of each of the types and subtypes of EGC [116]. In addition, by identifying the expression profile of cellular markers and the activity profile, it has been possible to characterize several EGC subpopulations [117, 118].

Depending on their location and morphology, EGC are nowadays grouped into four types. In 1994, Hanani and Reichenbach described intranodal type I or "protoplasmic enteric glia" and type II or "fibrous enteric glia", localized within bundles of nerve fibers [119]. In 2012, Gulbransen and Sharkey demonstrated the existence of two other types of EGC, type III or "enteric mucous glia", located in the subepithelial space, and type IV or "intramuscular enteric glia," closely linked to the nerve fibers of the muscle layers [118]. However, for the purpose of consistency, Gulbransen and Sharkey proposed a classification of EGC preferably linked to their location within the digestive tract. Thus, they identified EGC of the myenteric plexus, of the submucosal plexus, of the mucosa and intramuscular EGC. A subsequent study, distinguished three myenteric EGC subtypes based on differential response to adenosine triphosphate and suggested dynamic gene regulation in these cells [117]. Type I are described to have an integrative function of paracrine information from neurons and a function of modulating neuronal activity within the myenteric plexus [120]. Type II and III may reflect a more immature phenotype [117]. The expression of receptors, transmembrane channels or the combination of cell markers also differ between the different types of EGC. To date, no data explicitly confirms that different types of EGC have really distinct functional roles. Advanced technologies, such as single-cell RNA sequencing, are hopefully providing this information.

4.1.3 | Specific EGC markers

In mammals, the first described marker of mature EGC was the glial fibrillary acidic protein (GFAP) in 1980, an intermediate filament and a dynamic marker of EGC [121] (Figure 6). More specifically, its expression reflects the maturity of EGC and their reactivity [122]. Subsequently, in 1982, the intra-cytosolic calcium binding protein S100 was identified [123]. Even if GFAP and S100β have been for long considered reliable markers, their expression may change according to the status of EGC, that is proliferating or reactive glia, thus rendering challenging a systematic classification of these cells.

Other markers are nowadays available for the identification of adult EGC, such as transcription factors-2/8/9/10 with SRY domain (Sex determining region Y) (Sox 2/8/9/10) [111]. In particular, *Sox2* and *Sox10* genes encode for most frequently used EGC markers.

In 2015, Rao et al. identified a marker which was widely expressed by EGC in mice, called lipophilin or type 1 myelin proteolipid (PLP1), a fundamental structural protein of myelin [115]. In this study, S100β and PLP1 seemed suitable as markers of the enteric glial population, compared with GFAP. Indeed, $GFAP^{+}$ EGC were mainly found in the ganglia of the myenteric and submucosal plexus, therefore identifying intraganglionic EGC. Of note, glial markers appear at different times during embryonic development: S100β is expressed at E14.5 [123] while GFAP at E16.5 [121], Sox10 is already identified at E9.5 when ENCCs enter the gut [103], and PLP1 appears by E12.5 [91]. Because of the high plasticity of these cells and the species-related differences, taken individually, EGC markers do not unequivocally represent the entire glial population at any given (patho)physiological moment. To date, therefore, there is no real consensus regarding the use of a reliable pan-EGC marker [124].

5 | EGC POTENTIAL FOR ENGRAFTMENT IN BRAIN INJURY

Studies of enteric neurogenic tissue transplants are encouraging. Mention may in particular be made of those of Belkind-Gerson et al. [125]. Their work confirmed the feasibility of enteric neural stem cell (ENSC) transplantation into the brain parenchyma of brain injured rodent models. The realization of cell therapy by local or systemic route has shown survival of transplanted ENSCs, their proliferation and differentiation into neuronal and glial cells. In addition, transplanted cells were able to locally stimulate endogenous neurogenesis. One question arises: is it realistic to envisage comparable results for cell therapy using EGC in the setting of brain injury?

From the purely technical point of view, EGC are ideal candidates because they can be easily isolated and amplified from intestinal biopsies (Figure 6) [126]. The strength of the technique is the relative easiness to obtain pure cultures of EGC, without other cell (fibroblasts) contamination. Additionally, isolated cells can be cultured with a basic medium, without growth or other factors, which could make the system far from a physiological condition. Finally, it possible to amplify and store EGC. This is crucial when we need to perform a large panel of evaluations in cells obtained from a same patient. Specifically, EGC can be passaged up to five times, without changing their phenotype, and once frozen, 50%–60% of viable cells can be retrieved back in culture [120, 126, 127].

In conclusion, human EGC derived from a patient's own intestinal biopsy are proliferative, neurogenic, and non-immunogenic, without the need for genetic modification. They may be the most easily accessible neuronal precursor cell in the body, and their use in CNS and ENS transplantation is worth to investigating. Beyond the expected neurotrophic effects, several additional properties of EGC, namely the neurogenic potential, can be sought.

Trophic and neuroprotective effects

Within the ENS, neurons would exert a trophic dependence on EGC. This was clear in studies using animal models that had undergone EGC ablation, resulting in atrophy or even loss of neurons [128]. Like CNS glia, EGC regulate the formation, maintenance and function of neuronal synapses. In addition, in the case of injury of the ENS, Joseph et al. demonstrated a strong association between EGC and nerve fibers, allowing them to participate in axonal growth [7].

EGC exert a neuroprotective action by limiting oxidative stress, directly through the secretion of glutathione, an antioxidant molecule and indirectly, through 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) [129]. The latter, in turn, promotes the synthesis and secretion of glutathione. In addition, EGC also provide neuroprotection against excitotoxicity through the clearance of neuroactive substances, such as ATP and glutamate from the synaptic space [130].

There are many contiguous contacts between EGC and neurons, where the first intervene to modulate neuronal activity in different ways. On the one hand, they supply neurons with neurotransmitter precursors (i.e., glutamine), and secrete neuroactive mediators [131]. On the other hand, like astrocytes, they take part in synaptic functioning [132]. The excitability of EGC is manifested by the release of glutamate, making it possible to modulate synaptic transmission and neuronal excitability. In addition, potassium released into the extracellular milieu during neuronal activity is taken by transmembrane channels and transporters on EGC [133], limiting the deleterious effect of its accumulation on neuronal excitability and potentiating the postsynaptic action.

Immunomodulatory and inflammatory effects

Under physiological conditions, EGC participate in the immune homeostasis of the gastrointestinal wall [104]. In 2016, Kermarrec et al. demonstrated the immunomodulatory potential of EGC [134]. They revealed the inhibitory capacities of myenteric EGC on the immune response mediated by T lymphocytes, in vitro. One of the mechanisms involves the programmed cell death protein 1 (PD-1) receptor, expressed by T lymphocytes and its ligand (PD-L1), a glial cell surface protein [135]. Recent work showed that EGC interact with muscularis macrophages during intestinal inflammation (mouse model of colitis) and that this crosstalk, via connexin-43 signaling in EGC, mediates visceral pain [136].

In humans, EGC are involved in inflammatory enteropathies, such as Crohn's disease [137]. EGC respond to pro-inflammatory stimuli and actively participate in inflammatory processes [126, 138]. They secrete, for example, inflammatory mediators, including IL-1, and IL-6 [139]. During an inflammatory process, the expression of the binding protein S100β tends to increase [140, 141]. Like a pro-inflammatory cytokine, the latter modulates the signals of acute inflammation by EGC [126]. At nanomolar concentrations, S100β exhibits a neurotrophic effect, participating in the growth, survival and differentiation of neurons [142]. At micromolar concentrations, S100β is involved in neuroinflammatory and neurodegenerative processes in ENS [142]. The anti-inflammatory potential of EGC is also apprehended in the case of certain in vivo studies where inhibition of their activity induces deleterious effects. In 1998, Bush et al. set up a mouse model with ablation of $GFAP^+$ glial cells [128]. This resulted in increased permeability of the intestinal epithelium, induction of a severe inflammatory reaction, and hemorrhagic necrosis of the small intestine. At the cellular level, loss of EGC provoked neuronal atrophy of the myenteric plexus. The authors thus succeeded in demonstrating the important anti-inflammatory effects of EGC, for example, induced indirectly by the inhibition of the synthesis of pro-inflammatory factors. The importance of EGC in the maintaining of intestinal homeostasis has been recently confirmed in a more sophisticated study analyzing transcriptomes at the single-cell level to define the regulation of EGC heterogeneity in homeostasis and chronic inflammatory bowel disease [143]. In a very recent study, Progatzky et al. further confirm the role of EGC in tissue repair and immunity during inflammation [144]. By using a mouse model of helmint infection (*Heligmosomoides polygyrus*) in the gut, they revealed that EGC orchestrate an IFNγ-dependent immune response important for tissue repair and homeostasis maintenance during intestinal inflammation.

Neurogenic effects

In adult mammals, cell lines of the peripheral glia (Schwann cells, carotid body glia), can dedifferentiate and regain the properties of NCSC following stress tissue (lesion, infection, and tissue dissociation) [145]. As for EGC, recent studies have demonstrated an inducible neurogenic potential under similar conditions, such as acute inflammation, bacterial infection in vitro [7, 10]. In vivo, the study by Laranjeira et al. has revealed the neurogenic potential of EGC ($Sox10^+/GFAP^+$) in response to harmful chemical stimuli [9]. In rodent models, the authors demonstrated that EGC have a potential for transformation into NPCs. In addition, they observed, in vitro, that depending on the culture microenvironment, EGC are capable of giving rise to various subtypes of neurons. This work has also made it possible to reveal the functional competence of the newly formed neurons, which is characterized in particular by excitability and effective synapses. By exploring the characteristics and potentials of EGC, Laranjeira et al. also anticipated the interest of these cells for cell therapy in the context of enteric neuropathies. The specific mechanisms involved during

neurogenesis in vivo remain to be discovered. In 2009, Liu et al. have shown enteric neurogenesis in adult animals induced by the presence of serotonergic 5-hydroxytryptamine-4 receptor agonists [146].

Regarding the neurogenic and gliogenic potential of EGC, Joseph et al. evaluated the fate of specific CD49b^{+} EGC under normal conditions and then placed in the presence of different stimuli [7]. They thus demonstrated that neurogenesis and gliogenesis from these cells were detectable under physiological and pathological conditions. However, immunohistochemical analyses revealed low intensity neurogenesis, compared with gliogenesis, also under pathological conditions. This observation nevertheless constitutes further evidence of the neurogenic potential of EGC.

Subsequently, in 2017, Belkind-Gerson et al., provided additional details on this neurogenic and gliogenic potential, using transgenic mice. Immunohistochemical analyses revealed the existence of two subpopulations of EGC [10]. In response to inflammatory stimuli, one of these two subpopulations proliferate to maintain a constant the glial pool and the other is said to undergo "trans-differentiation," or neuronal differentiation. Constitutive neurogenesis in EGC is possible but is not initiated spontaneously. It is directed by all the environmental signals emitted by neurons, other EGC, the microbiota, and even immune cells [7, 9]. The neurogenic potential of myenteric EGC has been investigated in animals (adolescent mice) very recently by using single-cell analysis [11]. The pioneering results by Guyer et al., identified for the first time two specific glial subpopulations actively dividing, representing a pool of neuronal progenitors in the ENS. This finding confirms the existence of post-natal neurogenesis, which could have therapeutic application in ENS disorders. Specifically, two EGC populations were identified as functioning as reservoir neurogenic cells within the ENS. Intriguingly, analysis of transcription factors in the EGC population with neurogenic potential indicated Phox2b as driver of neurogenesis.

Furthermore, in the context of EGC transplantation, their neurogenic potential must be accompanied by the gliogenic potential in order to be able to be exploited in an optimal and sustainable manner. Indeed, neurons inevitably require the mechanical and functional support of glial cells to allow their proper functioning and survival.

EGC biosafety

The choice of cells to use in cell therapy should be based on the potential and beneficial properties that the cells can provide. Specifically, successful therapies require three conditions: (1) possibility of engraftment and long-term survival of the cells in the host; (2) safety of the grafted cells; and (3) structural and functional integration of the grafted within the host. Also, in the case of EGC, it is essential to recognize the health risks in the use of these cells. First, based on the high proliferative nature of isolated EGC, attention should be paid to their proliferation rate after engraftment. However, so far, no hyperproliferative glial disease has ever been described in the gut and human primary EGC show proliferative ability only in the presence of pro-inflammatory stimuli [126]. Then, in addition to immune rejection, neurodegenerative diseases likely to be transmitted and by EGC need to be considered. In vitro studies in human isolated EGC for MHC class II and MHC class I, have shown low protein expression [126], indicating that immunosuppression would not be necessary before cell engraftment. It is described that immunosuppressive therapy might lead to infection after stroke, which has a negative impact on (functional) outcomes. About neurodegeneration carried by EGC, a few scenarios need to be considered. First, Kreutfield-Jacob disease, a progressive and irreparably fatal neurodegenerative disease caused by the spread of pathological isoforms (PrP^{sc}) of the prion protein (PrP^{c}) [147]. The disease also affects astrocytes at an early stage, and the latter would be responsible for the transmission of the prion to the neuronal population [148]. Consequently, in an approach to extrapolating these observations, it is legitimate to wonder about the potential role of EGC as a prion vector. Recent studies have demonstrated the presence of replication sites for PrP^{c} protein within the myenteric plexus, preceding any replication at the CNS level [149]. However, there is no evidence regarding the presence and potential transmission of the PrP^{sc} by human EGC. In another situation, Parkinson's disease, a pathology characterized by the deposition of α-synuclein aggregates (Lewy bodies) in the neurons of the CNS, the involvement of EGC has been hypothesized. In 2003, Braak et al. proposed the early involvement of the ENS leading to diffusion to the CNS via preganglionic vagal fibers [150]. Subsequent studies in humans have shown Lewy bodies in the gastrointestinal tract during the pre-symptomatic stages. This hypothesis was reinforced by the observations of Shannon et al. in 2012, highlighting the presence of Lewy bodies in enteric neurons and EGC [151]. Conversely, recent studies in patients have not found any sign of the pathology (α-synuclein expression) in ENS neurons and EGC [152]. In addition, as for Alzheimer's disease (amyloid plaques), no direct transmission from EGC has been demonstrated in humans [92].

Finally, rat-derived EGC cell lines are currently classified as biosafety level 1 cells [153], therefore authorized to be handled in conventional animal facilities.

EGC-hydrogel biocompatibility

Having a good source of cells is not enough to have a successful therapeutic strategy. Before implantation, instead of grafting "free" cells, they could be seeded on a biomaterial that drives and supports tissue regeneration.

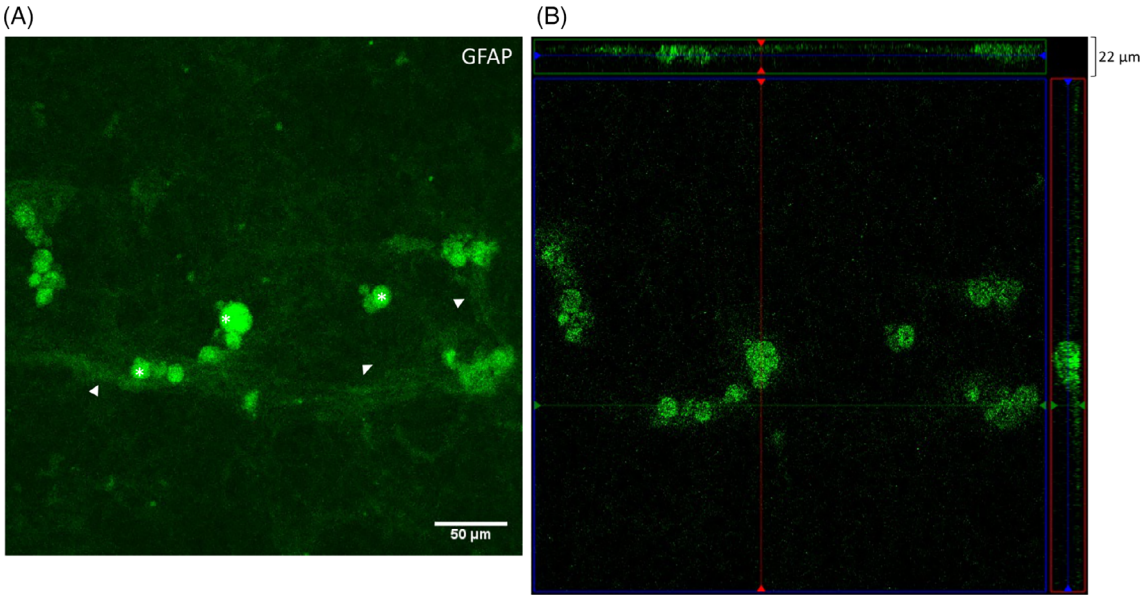

FIGURE 7 Immunostaining of EGC after seeding onto the hydrogel. (A) Sum slice projection from confocal z-stack images of EGC in Gal-C7 hydrogel after 7-days in culture. Staining with marker GFAP (green) shows the presence of the cell body (asterisk) and formation of connection between cells (arrowheads). EGC were identified using the mouse monoclonal anti-GFAP (1:1000, AbCam) with the secondary antibody goat anti-rabbit Alexa Fluor 488 (1:1000; Invitrogen). Scale bar: 50 µm. (B) The same field with orthogonal X (bottom) and Y (right) views showing EGC penetration into the hydrogel (thickness: 22 µm; 17 slices). Images were recorded using Zeiss LSM 710 confocal microscope (Cell Imaging Facility, INFINIty, Toulouse).

New biomaterials such as synthetic supramolecular non-polymer hydrogels may be interesting candidates in this field. Their softness facilitates cell growth in 3D, the purity of the molecules improves the reproducibility and the fibrous microstructure tends to stimulate and guide cell extensions. Here we present preliminary results from experiments combining glial cells and biomaterial (hydrogel) strategy, in the attempt to use it in future pre-clinical studies.

The field of supramolecular non-polymer gelling agents used as cell culture support is an emerging field (around 50 publications). The originality of this approach comes from the nature of the gelling agents. Gels are formed by the spontaneous self-assembly of molecules of low molar mass of well-defined molecular structure. Being synthetic, their composition is perfectly controlled. The self-assembly of these molecules gives gels that are mechanically weaker compared with polymer gels. It is an advantage for the cells of the nervous system. Also, the supramolecular fibers dissociates more or less easily depending on the molecular structure of the gelator, which allows to control their elimination. To date, the main supramolecular gelling agents used in cell culture are synthetic peptides. They may have a growing interest in neuronal regeneration [79, 154–156]. Besides, supramolecular non-polymer hydrogels derived from carbohydrates have been developed. They are synthesized more easily compared with currently marketed synthetic peptides, giving them a competitive advantage. Some of these carbohydrate gelators self-assemble in very large ribbons which withstand cell culture for few weeks. In vitro, these hydrogels have shown to stimulate the 3D growth and differentiation of human neural stem cells into neurons and glial cells. Neural cells assemble into 3D clusters connected by cellular extensions, guided by supramolecular fibers [79, 154, 156].

Compatibility between one of these hydrogels (*N*-heptyl-D-galactonamide) and primary isolated EGC was verified in the current study. *N*-heptyl-D-galactonamide hydrogel was prepared for cell culture as previously described [79]. Primary EGC isolated from the submucosal plexus of human colon biopsies [120, 126], cultured for 15 days and then amplified until passage 5 were prepared as previously described [126, 127, 153, 157]. EGC were then seeded on the *N*-heptyl-D-galactonamide hydrogel at a cell density of 75,000 cells/well ($n = 3$ experiments). Cell viability after 7 days of culture was realized and confirmed (data not shown). Robust 3D colonization and growth of EGC was observed at day 7 on the saccharide-derived supramolecular hydrogel (Figure 7). In these preliminary experiments, EGC demonstrated an excellent 3D integration in the supramolecular hydrogel, whose consistency is close to that of the brain.

6 | CONCLUSIONS

Regenerative medicine in brain injury appears very promising. Numerous preclinical studies and clinical trials attempt to identify the most satisfactory source and

type of cells. In this exploration, the ENS might find its place as a source of cells for carrying out transplants. Recent evidence demonstrates the presence of robust neurogenesis in the adult gut, with a remarkable rate of neural turnover, which maintains the number of enteric neurons constant. This has a great advantage compared with the limited neurogenesis in the CNS, and may drive profound biological and clinical implications. The ENS in the gut, differently to the CNS in the brain, has the second advantage to be easily accessible via routine biopsies (Figure 6). Within the ENS, EGC exhibit an interesting neurogenic and gliogenic potential. In the context of brain injuries, this ability has been tested for enteric neural progenitor cells. EGC engraftment should be verified in animal models, in a well-designed study to evaluate the structural and functional effects of long-term EGC engraftment, in vivo. An ideal study would assess the safety and efficacy of EGC in the aim to perform autologous transplantation in humans. In addition, it is possible to consider that an improvement in the survival and tissue integration of the transplanted cells is a guarantee of optimizing their effects. For this reason, a strategy combining EGC engraftment and the seeding on the hydrogel-based biomaterial appears very interesting. The advantages of EGC will be thus combined with hydrogel characteristics. Ideal characteristics for such biomaterial are: a well-defined composition, low cost, a fibrillar microstructure acting as a guide for the growth of neurites that could be possible to align, the permissiveness to cells to get 3D growth, the possibility to shape the gel by 3D printing, and a degradation not to fast which enables several weeks of development in vitro and/or in vivo. Such an approach has a strong potential to circumvent the restraints limiting the use of current therapies and might develop successful and safe interventions in patients.

AUTHOR CONTRIBUTIONS

Concept, *drafting of the manuscript and figures*: Sirine Hacene; *Experimental work*, *editing of the manuscript*: Alice Le Friec; *Editing of the manuscript*: Franck Desmoulin, Lorenne Robert, Nina Colitti; *Experimental work*, *drafting of the manuscript*, *obtaining funding*: Juliette Fitremann; *Draft and editing of the manuscript and figures*: Isabelle Loubinoux; *Concept and design*, *experimental work and analysis*, *draft and editing of the manuscript and figures*: Carla Cirillo.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. The data that support the findings of this study are available on request from the corresponding author.

ORCID

Carla Cirillo https://orcid.org/0000-0002-8445-5955

REFERENCES

1. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008;371(9624):1612–23.
2. Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, et al. Clinical outcomes of transplanted modified bone marrow–derived mesenchymal stem cells in stroke: A phase 1/2a study. Stroke. 2016;47(7):1817-24.
3. Mangin G, Kubis N. Cell therapy for ischemic stroke: how to turn a promising preclinical research into a successful clinical story. Stem Cell Rev Rep. 2019;15(2):176–93.
4. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol. 2012;9(5):286–94.
5. Metzger M, Bareiss PM, Danker T, Wagner S, Hennenlotter J, Guenther E, et al. Expansion and differentiation of neural progenitors derived from the human adult enteric nervous system. Gastroenterology. 2009;137(6):2063–73.
6. Kulkarni S, Micci M-A, Leser J, Shin C, Tang S-C, Fu Y-Y, et al. Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis. Proc Natl Acad Sci. 2017;114(18):E3709–18.
7. Joseph NM, He S, Quintana E, Kim YG, Núñez G, Morrison SJ. Enteric glia are multipotent in culture but primarily form glia in the adult rodent gut. J Clin Invest. 2011;121(9):3398–411.
8. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J, et al. Molecular architecture of the mouse nervous system. Cell. 2018;174(4):999–1014.
9. Laranjeira C, Sandgren K, Kessaris N, Richardson W, Potocnik A, Vanden Berghe P, et al. Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. J Clin Invest. 2011;121(9):3412–24.
10. Belkind-Gerson J, Graham HK, Reynolds J, Hotta R, Nagy N, Cheng L, et al. Colitis promotes neuronal differentiation of Sox2+ and PLP1+ enteric cells. Sci Rep. 2017;7(1):2525.
11. Guyer RA, Bhave S, Stavely R, Hotta R, Goldstein AM. Single-cell RNA sequencing reveals enteric glial subpopulations with therapeutic potential in hirschsprung disease. J Am Coll Surg. 2020;231:S213.
12. Holmstedt CA, Turan TN, Chimowitz MI. Atherosclerotic intracranial arterial stenosis: risk factors, diagnosis, and treatment. Lancet Neurol. 2013;12(11):1106–14.
13. Hoshino T, Sissani L, Labreuche J, Ducrocq G, Lavallée PC, Meseguer E, et al. Prevalence of systemic atherosclerosis burdens and overlapping stroke etiologies and their associations with long-term vascular prognosis in stroke with intracranial atherosclerotic disease. JAMA Neurol. 2018;75(2):203–11.
14. Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, et al. Ischaemic stroke. Nat Rev Dis Primers. 2019;5(1):70.
15. Muoio V, Persson PB, Sendeski MM. The neurovascular unit - concept review. Acta Physiol. 2014;210(4):790–8.
16. Figley CR, Stroman PW. The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals. Eur J Neurosci. 2011;33(4):577–88.
17. Duchemin S, Boily M, Sadekova N, Girouard H. The complex contribution of NOS interneurons in the physiology of cerebrovascular regulation. Front Neural Circuits. 2012;6:51.
18. Santello M, Calì C, Bezzi P. Gliotransmission and the tripartite synapse. Adv Exp Med Biol. 2012;970:307–31.
19. Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes regulate the blood-brain barrier. Nature. 2010;468(7323):557–61.
20. Choudhury GR, Ding S. Reactive astrocytes and therapeutic potential in focal ischemic stroke. Neurobiol Dis. 2016;85:234–44.
21. Thiel A, Cechetto DF, Heiss W-D, Hachinski V, Whitehead SN. Amyloid burden, neuroinflammation, and links to cognitive decline after ischemic stroke. Stroke. 2014;45(9):2825–9.
22. Loubinoux I, Kronenberg G, Endres M, Schumann-Bard P, Freret T, Filipkowski RK, et al. Post-stroke depression:

mechanisms, translation and therapy. J Cell Mol Med. 2012;16(9): 1961–9.
23. Rajan WD, Wojtas B, Gielniewski B, Gieryng A, Zawadzka M, Kaminska B. Dissecting functional phenotypes of microglia and macrophages in the rat brain after transient cerebral ischemia. Glia. 2019;67(2):232–45.
24. Xie L, Li W, Hersh J, Liu R, Yang SH. Experimental ischemic stroke induces long-term T cell activation in the brain. J Cereb Blood Flow Metab. 2018;39(11):2268–76.
25. Zrzavy T, Machado-Santos J, Christine S, Baumgartner C, Weiner HL, Butovsky O, et al. Dominant role of microglial and macrophage innate immune responses in human ischemic infarcts. Brain Pathol. 2018;28(6):791–805.
26. Cirillo C, Brihmat N, Castel-Lacanal E, Le Friec A, Barbieux-Guillot M, Raposo N, et al. Post-stroke remodeling processes in animal models and humans. J Cereb Blood Flow Metab. 2020; 40(1):3–22.
27. Kim SY, Hsu JE, Husbands LC, Kleim JA, Jones TA. Coordinated plasticity of synapses and astrocytes underlies practice-driven functional vicariation in peri-infarct motor cortex. J Neurosci. 2018;38(1):93–107.
28. Jones TA, Allred RP, Adkins DL, Hsu JE, O'Bryant A, Maldonado MA. Remodeling the brain with behavioral experience after stroke. Stroke. 2009;40(3):S136–8.
29. Luke LM, Allred RP, Jones TA. Unilateral ischemic sensorimotor cortical damage induces contralesional synaptogenesis and enhances skilled reaching with the ipsilateral forelimb in adult male rats. Synapse. 2004;54(4):187–99.
30. Takeuchi N, Izumi SI. Maladaptive plasticity for motor recovery after stroke: mechanisms and approaches. Neural Plast. 2012; 2012:359728.
31. Jones TA. Motor compensation and its effects on neural reorganization after stroke. Nat Rev Neurosci. 2017;18(5):267–80.
32. Thored P, Arvidsson A, Cacci E, Ahlenius H, Kallur T, Darsalia V, et al. Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem Cells. 2006; 24(3):739–47.
33. Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, et al. Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci. 2001;98(8):4710–5.
34. Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, et al. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell. 2002;110(4):429–41.
35. Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, et al. Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A. 2006;103(35):13198–202.
36. Nijs J, Meeus M, Versijpt J, Moens M, Bos I, Knaepen K, et al. Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: a new therapeutic target? Expert Opin Ther Targets. 2015;19(4):565–76.
37. Robinson RG, Spalletta G. Poststroke depression: a review. Can J Psychiatry. 2010;55(6):341–9.
38. Bernhardt J, Hayward KS, Kwakkel G, Ward NS, Wolf SL, Borschmann K, et al. Agreed definitions and a shared vision for new standards in stroke recovery research: the stroke recovery and rehabilitation roundtable taskforce. Int J Stroke. 2017;12(5): 444–50.
39. Kwakkel G, van Wegen EEH, Burridge JH, Winstein CJ, van Dokkum LEH, Alt Murphy M, et al. Standardized measurement of quality of upper limb movement after stroke: consensus-based core recommendations from the second stroke recovery and rehabilitation roundtable. Neurorehabil Neural Repair. 2019;33(11):951–8.
40. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. Neuron. 2010;67(2):181–98.
41. Vahidy FS, Rahbar MH, Zhu H, Rowan PJ, Bambhroliya AB, Savitz SI. Systematic review and meta-analysis of bone marrow-derived mononuclear cells in animal models of ischemic stroke. Stroke. 2016;47(6):1632–9.
42. Simonacci F, Bertozzi N, Grieco MP, Raposio E. From liposuction to adipose-derived stem cells: indications and technique. Acta Biomed. 2019;90(2):197–208.
43. Ikegame Y, Yamashita K, Hayashi SI, Mizuno H, Tawada M, You F, et al. Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy. 2011;13(6):675–85.
44. Tatsumi K, Ohashi K, Matsubara Y, Kohori A, Ohno T, Kakidachi H, et al. Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. Biochem Biophys Res Commun. 2013;431(2):203–9.
45. Kenmuir CL, Wechsler LR. Update on cell therapy for stroke. Stroke Vasc Neurol. 2017;2(2):59–64.
46. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–72.
47. Vu Q, Xie K, Eckert M, Zhao W, Cramer SC. Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. Neurology. 2014;82(14):1277–86.
48. Mangin G, Cogo A, Moisan A, Bonnin P, Maïer B, Kubis N. Intravenous administration of human adipose derived-mesenchymal stem cells is not efficient in diabetic or hypertensive micesubjected to focal cerebral ischemia. Front Neurosci. 2019;13:718.
49. Amer MH, Rose FRAJ, Shakesheff KM, Modo M, White LJ. Translational considerations in injectable cell-based therapeutics for neurological applications: concepts, progress and challenges. npj Regen Med. 2017;2(1):23.
50. Kojima T, Hirota Y, Ema M, Takahashi S, Miyoshi I, Okano H, et al. Subventricular zone-derived neural progenitor cells migrate along a blood vessel scaffold toward the post-stroke striatum. Stem Cells. 2010;28(3):545–54.
51. Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, et al. Human neural stem/progenitor cells, expanded in long-term neurosphere culture, promote functional recovery after focal ischemia in Mongolian gerbils. J Neurosci Res. 2004;78(2):215–23.
52. Fricker RA, Carpenter MK, Winkler C, Greco C, Gates MA, Björklund A. Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain. J Neurosci. 1999;19(14):5990–6005.
53. Rosado-de-Castro PH, Schmidt FR, Battistella V, Lopes de Souza SA, Gutfilen B, Goldenberg RC, et al. Biodistribution of bone marrow mononuclear cells after intra-arterial or intravenous transplantation in subacute stroke patients. Regen Med. 2013;8(2):145–55.
54. Park B-N, Shim W, Lee G, Bang OY, An Y-S, Yoon J-K, et al. Early distribution of intravenously injected mesenchymal stem cells in rats with acute brain trauma evaluated by 99mTc-HMPAO labeling. Nucl Med Biol. 2011;38(8):1175–82.
55. Ballester BR, Maier M, Duff A, Cameirão M, Bermúdez S, Duarte E, et al. A critical time window for recovery extends beyond one-year post-stroke. J Neurophysiol. 2019;122(1):350–7.
56. Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, et al. Transplantation of cultured human neuronal cells for patients with stroke. Neurology. 2000;55(4): 565–9.
57. Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. Cerebrovasc Dis. 2005;20(2):101–7.
58. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. Blood. 2005;105(7):2783–6.
59. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res. 2003;18(4):696–704.

60. Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA. Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system. Eur J Neurosci. 2004; 19(9):2388–98.
61. Zhang GL, Zhu ZH, Wang YZ. Neural stem cell transplantation therapy for brain ischemic stroke: review and perspectives. World J Stem Cells. 2019;11(10):817–30.
62. Savitz SI, Chopp M, Deans R, Carmichael T, Phinney D, Wechsler L, et al. Stem cell therapy as an emerging paradigm for stroke (STEPS) II. Stroke. 2011;42(3):825–9.
63. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke. 2009;40(6): 2244–50.
64. Wechsler LR. Stem cell therapies as an emerging paradigm in stroke (STEPS) bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. Stroke. 2009;40(2): 510–5.
65. Guan J, Zhu Z, Zhao RC, Xiao Z, Wu C, Han Q, et al. Transplantation of human mesenchymal stem cells loaded on collagen scaffolds for the treatment of traumatic brain injury in rats. Biomaterials. 2013;34(24):5937–46.
66. Accardo A, Cirillo C, Lionnet S, Vieu C, Loubinoux I. Interfacing cells with microengineered scaffolds for neural tissue reconstruction. Brain Res Bull. 2019;152:202–11.
67. Obermeyer JM, Ho E, Gracias A, Shoichet MS. Influencing neuroplasticity in stroke treatment with advanced biomaterials-based approaches. Adv Drug Deliv Rev. 2019;148:204–18.
68. Lacalle-Aurioles M, Cassel de Camps C, Zorca CE, Beitel LK, Durcan TM. Applying hiPSCs and biomaterials towards an understanding and treatment of traumatic brain injury. Front Cell Neurosci. 2020;14:1–14.
69. Tejeda G, Ciciriello AJ, Dumont CM. Biomaterial strategies to bolster neural stem cell-mediated repair of the central nervous system. Cells Tissues Organs. 2021;211(6):1–15.
70. Boisserand LSB, Kodama T, Papassin J, Auzely R, Moisan A, Rome C, et al. Biomaterial applications in cell-based therapy in experimental stroke. Stem Cells Int. 2016;2016:14.
71. Nih LR, Gojgini S, Carmichael ST, Segura T. Dual-function injectable angiogenic biomaterial for the repair of brain tissue following stroke. Nat Mater. 2018;17(7):642–51.
72. Nih LR, Sideris E, Carmichael ST, Segura T. Injection of microporous annealing particle (MAP) hydrogels in the stroke cavity reduces gliosis and inflammation and promotes NPC migration to the lesion. Adv Mater. 2017;29(32):1–8.
73. Ballios BG, Cooke MJ, Donaldson L, Coles BLK, Morshead CM, Van Der Kooy D, et al. A Hyaluronan-based injectable hydrogel improves the survival and integration of stem cell progeny following transplantation. Stem Cell Rep. 2015;4(6):1031–45.
74. Davoust C, Plas B, Béduer A, Demain B, Salabert A-S, Sol J-CC, et al. Regenerative potential of primary adult human neural stem cells on micropatterned bioimplants boosts motor recovery. Stem Cells Res Ther. 2017;8(1):253.
75. Vaysse L, Beduer A, Sol JC, Vieu C, Loubinoux I. Micropatterned bioimplant with guided neuronal cells to promote tissue reconstruction and improve functional recovery after primary motor cortex insult. Biomaterials. 2015;58:46–53.
76. Li A, Hokugo A, Yalom A, Berns EJ, Stephanopoulos N, McClendon MT, et al. A bioengineered peripheral nerve construct using aligned peptide amphiphile nanofibers. Biomaterials. 2014; 35(31):8780–90.
77. Sleep E, Cosgrove BD, McClendon MT, Preslar AT, Chen CH, Sangji MH, et al. Injectable biomimetic liquid crystalline scaffolds enhance muscle stem cell transplantation. Proc Natl Acad Sci U S A. 2017;114(38):E7919–28.
78. Álvarez Z, Kolberg-Edelbrock AN, Sasselli IR, Ortega JA, Qiu R, Syrgiannis Z, et al. Bioactive scaffolds with enhanced supramolecular motion promote recovery from spinal cord injury. Science. 2021;374(6569):848–56.
79. Chalard A, Vaysse L, Joseph P, Malaquin L, Souleille S, Lonetti B, et al. Simple synthetic molecular hydrogels from self-assembling alkylgalactonamides as scaffold for 3D neuronal cell growth. ACS Appl Mater Interfaces. 2018;10(20):17004–17.
80. Slotkin JR, Pritchard CD, Luque B, Ye J, Layer RT, Lawrence MS, et al. Biodegradable scaffolds promote tissue remodeling and functional improvement in non-human primates with acute spinal cord injury. Biomaterials. 2017;123:63–76.
81. Negoro T, Okura H, Maehata M, Hayashi S, Yoshida S, Takada N, et al. Trends in clinical trials for stroke by cell therapy: data mining ClinicalTrials.gov and the ICTRP portal site. npj Regen Med. 2019;4(1):20.
82. Kawabori M, Weintraub AH, Imai H, Zinkevych I, McAllister P, Steinberg GK, et al. Cell therapy for chronic TBI: interim analysis of the randomized controlled STEMTRA trial. Neurology. 2021; 96(8):e1202–14.
83. Jaillard A, Hommel M, Moisan A, Zeffiro TA, Favre-Wiki IM, Barbieux-Guillot M, et al. Autologous mesenchymal stem cells improve motor recovery in subacute ischemic stroke: a randomized clinical trial. Transl Stroke Res. 2020;11(5):910–23.
84. Kalladka D, Sinden J, Pollock K, Haig C, McLean J, Smith W, et al. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet. 2016; 388(10046):787–96.
85. Muir KW, Bulters D, Willmot M, Sprigg N, Dixit A, Ward N, et al. Intracerebral implantation of human neural stem cells and motor recovery after stroke: multicentre prospective single-arm study (PISCES-2). J Neurol Neurosurg Psychiatry. 2020;91(4): 396–401.
86. Hess DC, Wechsler LR, Clark WM, Savitz SI, Ford GA, Chiu D, et al. Safety and efficacy of multipotent adult progenitor cells in acute ischaemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Neurol. 2017;16(5): 360–8.
87. Bolan F, Louca I, Heal C, Cunningham CJ. The potential of biomaterial-based approaches as therapies for ischemic stroke: a systematic review and meta-analysis of pre-clinical studies. Front Neurol. 2019;10:924.
88. Kim KD, Lee KS, Coric D, Chang JJ, Harrop JS, Theodore N, et al. A study of probable benefit of a bioresorbable polymer scaffold for safety and neurological recovery in patients with complete thoracic spinal cord injury: 6-month results from the INSPIRE study. J Neurosurg Spine. 2021;34(5):808–17.
89. Bayliss WM, Starling EH. The movements and innervation of the small intestine. J Physiol. 1899;24(2):99–143.
90. Laranjeira C, Pachnis V. Enteric nervous system development: recent progress and future challenges. Auton Neurosci. 2009; 151(1):61–9.
91. Lasrado R, Boesmans W, Kleinjung J, Pin C, Bell D, Bhaw L, et al. Neurodevelopment: lineage-dependent spatial and functional organization of the mammalian enteric nervous system. Science. 2017;356(6339):722–6.
92. Gershon MD. The enteric nervous system: a second brain. Hosp Pract. 1999;34(7):31–52.
93. Kulkarni S, Ganz J, Bayrer J, Becker L, Bogunovic M, Rao M. Advances in enteric neurobiology: the "brain" in the gut in health and disease. J Neurosci. 2018;38(44):9346–54.
94. Uesaka T, Young HM, Pachnis V, Enomoto H. Development of the intrinsic and extrinsic innervation of the gut. Dev Biol. 2016; 417(2):158–67.
95. Young HM, Newgreen D. Enteric neural crest-derived cells: origin, identification, migration, and differentiation. Anat Rec. 2001; 262(1):1–15.
96. Heanue TA, Pachnis V. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. Nat Rev Neurosci. 2007;8(6):466–79.

97. Uesaka T, Nagashimada M, Enomoto H. Neuronal differentiation in Schwann cell lineage underlies postnatal neurogenesis in the enteric nervous system. J Neurosci. 2015;35(27):9879–88.
98. Espinosa-Medina I, Jevans B, Boismoreau F, Chettouh Z, Enomoto H, Müller T, et al. Dual origin of enteric neurons in vagal Schwann cell precursors and the sympathetic neural crest. Proc Natl Acad Sci U S A. 2017;114(45):11980–5.
99. Rao M, Gershon MD. Enteric nervous system development: what could possibly go wrong? Nat Rev Neurosci. 2018;19(9):552–65.
100. Hao MM, Capoccia E, Cirillo C, Boesmans W, Berghe PV. Arundic acid prevents developmental upregulation of S100B expression and inhibits enteric glial development. Front Cell Neurosci. 2017; 11:42.
101. Young HM, Hearn CJ, Farlie PG, Canty AJ, Thomas PQ, Newgreen DF. GDNF is a chemoattractant for enteric neural cells. Dev Biol. 2001;229(2):503–16.
102. Barlow A, De Graaff E, Pachnis V. Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron. 2003;40(5):905–16.
103. Kim J, Lo L, Dormand E, Anderson DJ. SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. Neuron. 2003;38(1):17–31.
104. Vergnolle N, Cirillo C. Neurons and glia in the enteric nervous system and epithelial barrier function. Phys Ther. 2018;33(4): 269–80.
105. Bornstein JC, Hendriks R, Furness JB, Trussell DC. Ramifications of the axons of AH-neurons injected with the intracellular marker biocytin in the myenteric plexus of the Guinea pig small intestine. J Comp Neurol. 1991;314(3):437–51.
106. Nurgali K, Stebbing MJ, Furness JB. Correlation of electrophysiological and morphological characteristics of enteric neurons in the mouse colon. J Comp Neurol. 2004;468(1):112–24.
107. Furness JB. Novel gut afferents: intrinsic afferent neurons and intestinofugal neurons. Auton Neurosci. 2006;125(1-2):81–5.
108. Carbone SE, Jovanovska V, Nurgali K, Brookes SJH. Human enteric neurons: morphological, electrophysiological, and neurochemical identification. Neurogastroenterol Motil. 2014;26(12): 1812–6.
109. Knowles CH, Veress B, Kapur RP, Wedel T, Farrugia G, Vanderwinden JM, et al. Quantitation of cellular components of the enteric nervous system in the normal human gastrointestinal tract—report on behalf of the Gastro 2009 International Working Group. Neurogastroenterol Motil. 2011;23(2):115–24.
110. Gabella G. Ultrastructure of the nerve plexuses of the mammalian intestine: the enteric glial cells. Neuroscience. 1981;6(3):425–36.
111. Hoff S, Zeller F, Von Weyhern CWH, Wegner M, Schemann M, Michel K, et al. Quantitative assessment of glial cells in the human and Guinea pig enteric nervous system with an anti-Sox8/9/10 antibody. J Comp Neurol. 2008;509(4):356–71.
112. Komuro T, Baluk P, Burnstock G. An ultrastructural study of neurons and non-neuronal cells in the myenteric plexus of the rabbit colon. Neuroscience. 1982;7(7):1797–806.
113. Rühl A, Nasser Y, Sharkey KA. Enteric glia. Neurogastroenterol Motil. 2004;16(SUPPL. 1):44–9.
114. Gabella G. Fine structure of the myenteric plexus in the Guinea-pig ileum. J Anat. 1972;111(Pt 1):69–97.
115. Rao M, Nelms BD, Dong L, Salinas-Rios V, Rutlin M, Gershon MD, et al. Enteric glia express proteolipid protein 1 and are a transcriptionally unique population of glia in the mammalian nervous system. Glia. 2015;63(11):2040–57.
116. Le Douarin N, Dulac C, Dupin E, Cameron-Curry P. Glial cell lineages in the neural crest. Glia. 1991;4(2):175–84.
117. Boesmans W, Lasrado R, Vanden Berghe P, Pachnis V. Heterogeneity and phenotypic plasticity of glial cells in the mammalian enteric nervous system. Glia. 2015;63(2):229–41.
118. Gulbransen BD, Sharkey KA. Novel functional roles for enteric glia in the gastrointestinal tract. Nat Rev Gastroenterol Hepatol. 2012;9(11):625–32.
119. Hanani M, Reichenbach A. Morphology of horseradish peroxidase (HRP)-injected glial cells in the myenteric plexus of the Guinea-pig. Cell Tissue Res. 1994;278(1):153–60.
120. Boesmans W, Cirillo C, Van den Abbeel V, Van den Haute C, Depoortere I, Tack J, et al. Neurotransmitters involved in fast excitatory neurotransmission directly activate enteric glial cells. Neurogastroenterol Motil. 2013;25(2):e151–60.
121. Jessen KR, Mirsky R. Glial cells in the enteric nervous system contain glial fibrillary acidic protein. Nature. 1980;286(5774):736–7.
122. Von Boyen GBT, Steinkamp M, Geerling I, Reinshagen M, Schäfer KH, Adler G, et al. Proinflammatory cytokines induce neurotrophic factor expression in enteric glia: a key to the regulation of epithelial apoptosis in Crohn's disease. Inflamm Bowel Dis. 2006;12(5):346–54.
123. Ferri GL, Probert L, Cocchia D, Michetti F, Marangos PJ, Polak JM. Evidence for the presence of S-100 protein in the glial component of the human enteric nervous system. Nature. 1982; 297(5865):409–10.
124. Grubišić V, Gulbransen BD. Enteric glia: the most alimentary of all glia. J Physiol. 2017;595(2):557–70.
125. Belkind-Gerson J, Hotta R, Whalen M, Nayyar N, Nagy N, Cheng L, et al. Engraftment of enteric neural progenitor cells into the injured adult brain. BMC Neurosci. 2016;17(1):5.
126. Cirillo C, Sarnelli G, Turco F, Mango A, Grosso M, Aprea G, et al. Proinflammatory stimuli activates human-derived enteroglial cells and induces autocrine nitric oxide production. Neurogastroenterol Motil. 2011;23(9):10–2.
127. Soret R, Coquenlorge S, Cossais F, Meurette G, Rolli-Derkinderen M, Neunlist M. Characterization of human, mouse, and rat cultures of enteric glial cells and their effect on intestinal epithelial cells. Neurogastroenterol Motil. 2013;25(11):e755–64.
128. Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, et al. Fulminant jejuno-ileitis following ablation of enteric gila in adult transgenic mice. Cell. 1998;93(2):189–201.
129. Abdo H, Mahé MM, Derkinderen P, Bach-Ngohou K, Neunlist M, Lardeux B. The omega-6 fatty acid derivative 15-deoxy-Δ12,14-prostaglandin J2 is involved in neuroprotection by enteric glial cells against oxidative stress. J Physiol. 2012; 590(11):2739–50.
130. Fletcher EL, Clark MJ, Furness JB. Neuronal and glial localization of GABA transporter immunoreactivity in the myenteric plexus. Cell Tissue Res. 2002;308(3):339–46.
131. Nagahama M, Semba R, Tsuzuki M, Aoki E. L-arginine immunoreactive enteric glial cells in the enteric nervous system of rat ileum. Neurosignals. 2001;10(5):336–40.
132. Porter JT, McCarthy KD. Astrocytic neurotransmitter receptors in situ and in vivo. Prog Neurobiol. 1997;51(4):439–55.
133. Walz W. Chloride/anion channels in glial cell membranes. Glia. 2002;40(1):1–10.
134. Kermarrec L, Durand T, Neunlist M, Naveilhan P, Neveu I. Enteric glial cells have specific immunosuppressive properties. J Neuroimmunol. 2016;295-296:79–83.
135. Yamane H, Isozaki H, Takeyama M, Ochi N, Kudo K, Honda Y, et al. Programmed cell death protein 1 and programmed death-ligand 1 are expressed on the surface of some small-cell lung cancer lines. Am J Cancer Res. 2015;5(4):1553–7.
136. Grubišić V, McClain JL, Fried DE, Grants I, Rajasekhar P, Csizmadia E, et al. Enteric glia modulate macrophage phenotype and visceral sensitivity following inflammation. Cell Rep. 2020; 32(10):108100.
137. Cornet A, Savidge TC, Cabarrocas J, Deng WL, Colombel JF, Lassmann H, et al. Enterocolitis induced by autoimmune targeting of enteric glial cells: a possible mechanism in Crohn's disease? Proc Natl Acad Sci U S A. 2001;98(23):13306–11.

138. Turco F, Sarnelli G, Cirillo C, Palumbo I, De Giorgi F, D'Alessandro A, et al. Enteroglial-derived S100B protein integrates bacteria-induced toll-like receptor signalling in human enteric glial cells. Gut. 2014;63(1):105–15.
139. Murakami M, Ohta T, Ito S. Lipopolysaccharides enhance the action of bradykinin in enteric neurons via secretion of interleukin-1β from enteric glial cells. J Neurosci Res. 2009;87(9): 2095–104.
140. Cirillo C, Sarnelli G, Esposito G, Grosso M, Petruzzelli R, Izzo P, et al. Increased mucosal nitric oxide production in ulcerative colitis is mediated in part by the enteroglial-derived S100B protein. Neurogastroenterol Motil. 2009;21(11):1209.
141. Esposito G, Cirillo C, Sarnelli G, De Filippis D, D'Armiento FP, Rocco A, et al. Enteric glial-derived S100B protein stimulates nitric oxide production in celiac disease. Gastroenterology. 2007; 133(3):918–25.
142. Huttunen HJ, Kuja-Panula J, Sorci G, Agneletti AL, Donato R, Rauvala H. Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. J Biol Chem. 2000; 275(51):40096–105.
143. Baghdadi MB, Ayyaz A, Coquenlorge S, Chu B, Kumar S, Streutker C, et al. Enteric glial cell heterogeneity regulates intestinal stem cell niches. Cell Stem Cell. 2021;29(1):86–100.e6.
144. Progatzky F, Shapiro M, Chng SH, Garcia-Cassani B, Classon CH, Sevgi S, et al. Regulation of intestinal immunity and tissue repair by enteric glia. Nature. 2021;599:125–30.
145. McCallum S, Obata Y, Fourli E, Boeing S, Peddie CJ, Xu Q, et al. Enteric glia as a source of neural progenitors in adult zebrafish. Elife. 2020;9:1–66.
146. Liu MT, Kuan YH, Wang J, Hen R, Gershon MD. 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. J Neurosci. 2009;29(31):9683–99.
147. Kovacs GG, Budka H. Prion diseases: from protein to cell pathology. Am J Pathol. 2008;172(3):555–65.
148. Victoria GS, Arkhipenko A, Zhu S, Syan S, Zurzolo C. Astrocyte-to-neuron intercellular prion transfer is mediated by cell-cell contact. Sci Rep. 2016;6:20762.
149. Kujala P, Raymond CR, Romeijn M, Godsave SF, van Kasteren SI, Wille H, et al. Prion uptake in the gut: identification of the first uptake and replication sites. PLoS Pathog. 2011;7(12): e1002449.
150. Braak H, De Vos RAI, Bohl J, Del Tredici K. Gastric α-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett. 2006;396(1):67–72.
151. Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord. 2012; 27(6):709–15.
152. Desmet A-S, Cirillo C, Tack J, Vandenberghe W, Berghe PV. Live calcium and mitochondrial imaging in the enteric nervous system of parkinson patients and controls. Elife. 2017;6:e26850.
153. Rühl A, Trotter J, Stremmel W. Isolation of enteric glia and establishment of transformed enteroglial cell lines from the myenteric plexus of adult rat. Neurogastroenterol Motil. 2001; 13(1):95–106.
154. Chalard A, Joseph P, Souleille S, Lonetti B, Saffon-Merceron N, Loubinoux I, et al. Wet spinning and radial self-assembly of a carbohydrate low molecular weight gelator into well organized hydrogel filaments. Nanoscale. 2019;11(32):15043–56.
155. Somaa FA, Wang TY, Niclis JC, Bruggeman KF, Kauhausen JA, Guo H, et al. Peptide-based scaffolds support human cortical progenitor graft integration to reduce atrophy and promote functional repair in a model of stroke. Cell Rep. 2017;20(8):1964–77.
156. Andriamiseza F, Bordignon D, Payré B, Vaysse L, Fitremann J. 3D printing of biocompatible low molecular weight gels: imbricated structures with sacrificial and persistent N-alkyl-d-galactonamides. J Colloid Interface Sci. 2022;617:156–70.
157. Rühl A, Franzke S, Collins SM, Stremmel W. Interleukin-6 expression and regulation in rat enteric glial cells. Am J Physiol Gastrointest Liver Physiol. 2001;280:G1163–71.

How to cite this article: Hacene S, Le Friec A, Desmoulin F, Robert L, Colitti N, Fitremann J, et al. Present and future avenues of cell-based therapy for brain injury: The enteric nervous system as a potential cell source. Brain Pathology. 2022;32(5):e13105. https://doi.org/10.1111/bpa.13105