Cell therapy rejuvenates the neuro-glial-vascular unit

Bandy Chen*

The rise of the aging population parallels the rapidly increasing cases of neurological disorders. This puts pressure on scientists and physicians to find novel methods that can prevent and treat neurodegeneration. The brain is made up of a complex network of different cell types that work in tandem to maintain systemic homeostasis. These cells include vascular cells (endothelial cells, pericytes, and smooth muscle cells), glial cells (astrocytes, microglia, and oligodendrocytes), and neurons that have different functions to complement each other and form the neuro-glial-vascular unit (NVU). These elements act in concert to orchestrate neurovascular coupling and maintain blood–brain barrier (BBB) integrity. Unlike other systems in the human body, the brain has limited regenerative capacity. To overcome this limitation, novel approaches in stem cell biology, immune cell engineering, and bioengineering work in tandem to repair, replace, and restore function in the central nervous system. Due to the diverse cell types of the central nervous system, cell therapy allows cell type-specific modifications to precisely target neural circuitries and advance personalized medicine. This puts cell therapy at the forefront as a potential treatment to rejuvenate the cerebral landscape. This perspective focuses on the impact of cell therapy through the lens of the NVU.

To understand the cerebral landscape, induced pluripotent stem cells (iPSCs) have emerged as a powerful tool to model the brain in health and disease. A prominent example is the development of a human BBB chip by combining organ-chip technology and human iPSC-derived tissue (Vatine et al., 2019). Using iPSCs to mimic components of the NVU such as brain microvascular endothelial cells, astrocytes, and pericytes in a human chip, the iPSC-based BBB-Chip demonstrates physiological transendothelial electrical resistance and predicts central nervous system penetrance of pharmacologics. To develop a platform for modeling neurological disorders, patient-derived iPSCs can be utilized to create a personalized BBB-Chip that encompasses a diseased NVU. The patient-specific BBB-Chip recapitulates disease-specific defects such as lack of transporters and disruption of BBB integrity. For example, Huntington's disease iPSC-derived BBB-Chips demonstrate a significant increase in dextran-FITC molecule permeability compared to healthy control iPSC-derived BBB-Chips. This revolutionary technique not only provides a comparison of the neurovascular landscape in different disease models, but also allows elucidation of interindividual variability in the BBB performance of healthy and diseased patients. The utilization of cell modeling to advance drug screening and personalized medicine is the first step of cell therapy.

Remodeling of the neurovasculature: Cell therapy has primarily focused on the peripheral vasculature due to the accessibility (absence of the BBB), lower risk of complications (less sensitive to immune responses), and greater regenerative capacity. Despite these challenges, recent efforts have focused on enhancing vascular plasticity in the brain. The neurovasculature is involved in the regulation of various brain systems such as BBB integrity, cerebrospinal fluid (CSF) dynamics, myelination, and neuronal activity. Cerebrovascular network remodeling is seen in healthy aging and neurodegeneration. Cell therapy focused on maintaining the NVU and vascular function by rejuvenating specific brain systems has been a primary focus in neurological care (**Figure 1**). Stroke is the primary cause of long-term disability and the second leading cause of death globally. Current treatments for stroke primarily focus on the hyperacute phase for a favorable therapeutic effect. This adds urgency to the development of regenerative therapies for subacute and chronic phases. In a mouse model of chronic cerebral infarction, intracerebral administration of bone marrow-derived mononuclear cells improves motor function through the recovery of cerebral blood flow (Kitamura et al., 2023). The affected brain area displayed increased angiogenesis and attenuated astrogliosis and microgliosis. However, the viability of grafted cells in the ischemic brain limits the potential therapeutic benefits. Co-treatment with bone marrow mesenchymal stem cells (MSCs) and insulin-like growth factor-1 improves cerebral blood flow and behavioral outcomes after ischemia compared to MSC treatment alone (Shen et al., 2021). Insulin-like growth factor-1 protects MSCs from apoptosis and promotes cell migration, thereby increasing angiogenesis and neurogenesis. The proposed mechanism involves insulin-like growth factor-1-mediated upregulation of angiogenic factors brain-derived neurotrophic factor, vascular endothelial growth factor, and Ang-1. In a rat model of transient cerebral ischemia, MSCs engineered to overexpress vascular endothelial growth factor display greater neuroprotective effects such as increased angiogenesis, improved BBB integrity, and reduced neuronal degeneration and infarct volume than the MSC-control group (Liu et al., 2023). Overall, bone marrow-derived cells demonstrate the potential to treat the different phases of stroke by promoting cerebral blood flow, vascular plasticity, and glial quiescence. It is of great interest to explore cell therapies utilized for vascular diseases in the context of other neurological diseases due to the intimate link between the neurovasculature and other processes of the brain such as the glymphatic system.

Leveraging the glymphatic system: The glymphatic system is the primary waste clearance system of the brain with aquaporin-4 (AQP4) on astrocytic endfeet being the main regulator of CSF dynamics. Alterations in glymphatic function are seen in various neurological disorders with early manifestations, making the glymphatic system an ideal target for cell therapy. For example, in the BACHD mice (a model of Huntington's disease), there is a downregulation of AQP4 with elevated astrogliosis compared to wild-type mice (Wu et al., 2020). The glymphatic dysfunction decreases the therapeutic efficiency of antisense oligonucleotides (ASOs) utilized to suppress huntingtin production due to attenuated brain distribution of ASOs. A combined intravenous injection of MSCs and ASOs enhances ASO distribution efficiency and reduces mutant huntingtin levels. This is attributed to MSC-induced AQP4 regeneration, resulting in improved glymphatic flow and reduced astrogliosis. Cerebral small-vessel disease is the main cause of vascular cognitive impairment with the accumulation of amyloid-β (Aβ) associated with disease severity. Glymphatic dysfunction is believed to be a main contributor to Aβ deposition, increasing the risk for cerebral small-vessel disease and other neurological disorders. In the stroke-prone renovascular hypertensive rat (a model of cerebral small-vessel disease), there is increased cognitive impairment, white matter lesions, Aβ deposition, and loss of AQP4 polarity (Liu et al., 2022). Treatment with MSCs improves cognitive function and reduces Aβ deposition and neuroinflammation by promoting AQP4 polarity. Lastly, transplantation of MSCs into the lateral ventricle of hydrocephalus mice results in the upregulation of AQP4 to enhance clearance of periventricular edema (Garcia-Bonilla et al., 2023). This is accompanied by an increase in neuroprotective factors such as nerve growth factor, vascular endothelial growth factor, hypoxia-inducible factor 1-alpha, and transforming growth factor β1. While these studies demonstrate MSC-induced restoration of AQP4

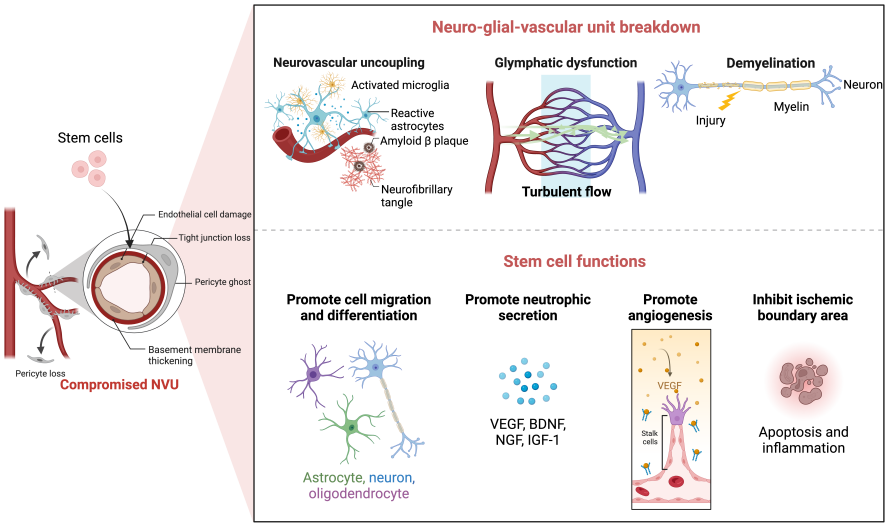

Figure 1 | **Rejuvenation of the neuro-glial-vascular unit.**
The breakdown of the neuro-glial-vascular unit leads to a domino effect of neurodegeneration including neurovascular uncoupling, glymphatic dysfunction, and demyelination. Current cell therapy can be utilized to target these different processes individually. The next progression in the field is the utilization of combinational therapies to target multiple processes at once to maximize therapeutic efficacy. Created with BioRender.com. BDNF: Brain-derived neurotrophic factor; IGF-1: insulin-like growth factor 1; NGF: nerve growth factor; VEGF: vascular endothelial growth factor.

polarity, the mechanisms of glymphatic rewiring remain to be elucidated. Additional research on the downstream effects of improving glymphatic flow on vascular remodeling, myelination, and neuronal activity can help explain the benefits on cognitive function.

Enhancing myelin regeneration: The NVU and glymphatic system are intimately linked and work in tandem to maintain proper myelination and neural conduction. Demyelination is a prominent phenotype in many neurological disorders, encouraging the development of remyelination therapies. Multiple sclerosis is the most common demyelinating disease that leads to progressive disability. Therapies to enhance remyelination have been unsuccessful due to intrinsic and extrinsic inhibition of oligodendrocyte progenitor cell (OPC) function and differentiation. A strategy aimed at enhancing OPC function to overcome the negative environment and increase survivability is through CRISPR/Cas9 technology. For example, in the local demyelinated lesions, there are high levels of Semaphorin 3A that act as a chemorepulsive factor for OPCs by binding to neuropilin-1 (NRP1) and reducing remyelination. To address this limitation, the NRP1 receptor on human embryonic stem cell-derived OPCs has been deleted using CRISPR/Cas9 technology (Wagstaff et al., 2024). In mice, transplantation of $NRP1^{-/-}$ OPCs in focal demyelinating lesions enhances migration and remyelination. This therapy works equally well in young and aged mice, overcoming the extrinsic aging environment often seen in human demyelinating diseases. In a rat model of repetitive traumatic brain injury, treatment with MSCs attenuates neurobehavioral disorders, white matter disintegration, and neuroinflammation by modulating microglial responses (Wang et al., 2024). MSCs-mediated paracrine factors reduce over-accumulation of tumor necrosis factor-α containing microglia and stimulate neurotrophic factors, which promote an anti-inflammatory state for recovery. Attenuating excessive accumulation of proinflammatory cytokines in demyelinated lesions allows greater neuron survivability and recruitment of OPCs for remyelination. Whether cell therapy-mediated remyelination can adequately restore neuronal activity and functional connectivity needs further exploration.

Rewiring neural circuitry to recover neuronal activity: The breakdown of the NVU and its associated processes including CSF dynamics and myelination leads to a domino effect of degeneration that contributes to the rewiring of the neural circuitry and eventual loss of functional connectivity. One example is Parkinson's disease, characterized by a loss of dopaminergic neurons in the substantia nigra and is a prominent neurological disorder being targeted by cell therapy. Initial studies demonstrate the potential of implanting dopaminergic neurons from human iPSCs and embryonic stem cells to replace neuronal loss and rewire the nigrostriatal pathway. In the 6-OHDA mouse model of Parkinson's disease, transplantation of human embryonic stem cells-derived midbrain dopamine or cortical glutamate neurons into the substantia nigra or striatum demonstrates extensive graft integration with host circuitry (Xiong et al., 2021). Both neuronal types project over a long distance to different targets, indicating that pathfinding and target projection are determined by the identity of the neurons. Interestingly, anatomical presynaptic inputs are predominately determined by graft location, while the functional synaptic inputs are determined by the identity of the neurons. This highlights the importance of transplanting enriched, fated neural progenitors to achieve specific circuitry reconstruction. Due to the limited capacity for long-distance axonal growth, cells are often transplanted in the striatum to replenish local dopamine. This means that several dopaminergic pathways are not re-instated and may underlie the partial restoration of motor function. To address this limitation, a combined cell and gene therapy approach can be utilized to achieve functional and anatomically precise reconstruction of long-distance circuitry. Injection of homotopic midbrain dopamine graft with viral delivery of glial cell line-derived neurotrophic factor to the striatum facilitates a dense striatal innervation, re-instatement of striatal dopamine levels, and correction of motor function comparable to ectopic grafts placed directly into the striatal target (Moriarty et al., 2022). However, the survivability of transplanted neurons and the proliferation of unwanted cell populations significantly decrease the efficacy of therapeutic potentials. These limitations can be offset by the co-transplantation of autologous regulatory T cells (T_{reg}). In mice, intra-striatal co-transplantation of T_{reg} and iPSC-derived dopaminergic neurons significantly increases the survival rate of implanted dopaminergic neurons and suppresses the proliferation of non-dopaminergic neurons (Park et al., 2023). In response to needle trauma, the brain triggers an inflammatory response with local release of pro-inflammatory cytokines and activation of microglia and astrocytes. When prolonged, the uncontrolled inflammation can lead to neuronal damage of both endogenous and implanted cells. Co-injection of Treg ameliorates the inflammatory response by directly acting on dopaminergic neurons and indirectly acting on surrounding resident brain cells. This is a prime example of cell therapy affecting multiple components of the NVU to enact compounding effects. It is imperative for the field to leverage combinational therapies to maximize therapeutic targets and efficacy and patient outcomes.

Conclusions: The ability of cell therapy to impact different brain processes such as vascular modeling, CSF dynamics, myelination, and neuronal activity hints at the potential to be at the forefront of neurological care. Due to the intimate link between the various processes, it is crucial for studies to focus on multiple processes for therapeutic outcomes in hopes of advancing combinational therapy. Currently, limitations of cell therapy include delivery challenges, tumorigenicity, immune rejection, and survival/differentiation. Further investigation on the temporal specificity of cell therapy is needed to understand the impact of the aging environment on therapeutic efficacy and to distinguish between promotion, prevention, and treatment interventions.

Bandy Chen*
Department of Medicine, UC San Diego School of Medicine, La Jolla, CA, USA
***Correspondence to:** Bandy Chen, bac008@health.ucsd.edu.
https://orcid.org/0009-0009-9099-1218 (Bandy Chen)

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