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Human Neural Stem Cell Perilesional Transplant Potential Recovery in Penetrating Traumatic Brain Injury

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Abstract— **Traumatic brain injury (TBI) is a leading cause of death and disability in the United States [1-5] Despite exceeding the death rate of cancer by 3.5 times, there is inadequate treatment that directly targets the TBI lesion, in particular the lesions due to penetrating traumatic brain injury (pTBI)[1]; pTBI is a niche area of TBI injuries that focuses on a foreign object entering and harming the brain [2]. Since the discovery of neural stem cells in the subventricular zone (SVZ) and dentate gyrus (DG), exploration of transplantation treatment has become a topic of interest [6-8] The aim of this study was to understand how stem cell treatments could be a optimized to address penetrating traumatic brain injury. It was initially thought that neural cells were non regenerative in central nervous system (CNS) injuries and that adult neurogenesis is limited in the SVZ and DG. However, neural stem cells are still present within the subventricular cortex after the injury. This demonstrates how transplanting endogenous cells could be a better treatment option in comparison to the current treatments that only mitigate secondary injuries and symptoms[3,9,10] Indeed, a growing number of experiments and animal trials have shown that human neural stem cells (hNSCs) transplanted perilesional to the cavity have the potential to aid pTBI recovery [2,5,11,12].**

I. INTRODUCTION

In the United States, around 1.7 million TBI cases are reported each year [13]. Specifically, pTBI contributes to the majority of firearm deaths [13]. That is about 20,000 headshots occur annually, and 70% of severe blast injuries result from pTBI [4,14]. Often pTBI leaves its patients with a lower life quality and long term disabilities such as Alzheimer's Disease, seizures, and neuroendocrine dysregulation. It also poses an economic burden costing $76.5 billion dollars for both indirect (loss in the workforce, emotional, psychosocial burdens, etc) and direct (emergency treatment, hospitalization, healthcare, etc.) expenses per year [13]. Despite this, there are presently no effective treatment methods for pTBI as current treatment of pTBI is primarily focused on managing secondary injury and symptoms [2,5,9,11,15].Therefore there is a need for more effective treatment methods that target the pTBI lesions directly. That is, therapies that are aimed at replacing the lost neurons within the resulting brain cavity.

Human neural stem cells hNSCs and their potential to promote proliferation (the increase in the number of such cells as a result of cell growth and division) and differentiation (a process in which young, unspecialized cells inhibit individual characteristics from their environment and adopt a specialized function and form) within the resulting cavity, explicitly in the procephalic cyst, reveals a compelling future treatment option for the most important consequence of pTBI: neuronal loss [1,2,5-7,9,11,15,16,19-21]. Neurologists have demonstrated evidence of proliferation, differentiation, engraftment (growth of transplanted cells and successful interaction with new environment), reduced inflammation, and improvement of motor and cognitive deficits post hNSC transplant [2,5,9,11,15].

To explore how hNSCs could be an improved treatment option for the lost neurons in pTBI, primary and review journals demonstrating existing model hNSC transplantation were examined. Twenty plus journals from sources, such as PubMed, fell within the last decade of research. Key search words incorporated in the research process included perilesional, hNSC, pTBI, transplant, degeneration, and TBI in general. After organizing the data in accordance to the paper outline, original figures were curated utilizing Biorender. While research and primary experiments on the subject are limited, present data suggests that hNSCs may be a viable treatment option for pTBI.

II. PENETRATING TRAUMATIC BRAIN INJURY

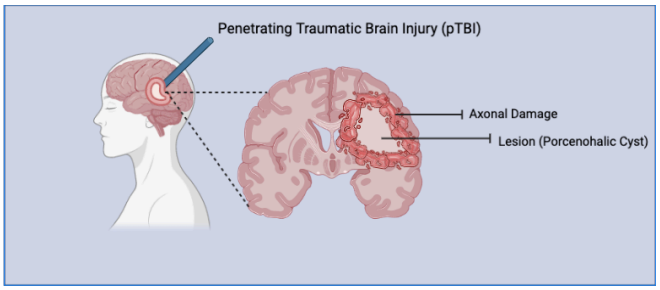

Figure 1: Representative of a probe or projectile penetrating the brain. Surrounding the lesion illustrates secondary damage at the cellular level, such as apoptosis, axonal damage, demyelination, and the formation of a porencephalic cyst (created using Biorender).

pTBI is defined as when an object breaches the skull, dura, and damages the parenchyma. Roughly, pTBI includes all traumatic brain injuries other than blunt head trauma (see Figure 1) [2]. Generally, when the projectile travels through the brain parenchyma, it causes a transient sonic wave which crushes the soft brain tissue and cultivates a permanent track of injury [3]. The severity of pTBI is heavily dependent on the velocity of the object at the point of penetration [3,6]. High velocity penetration consists of injuries produced by bullets or

shell fragments from direct trauma or shockwave injury surrounding brain tissue. On the other hand, low velocity penetration derives from sharp objects, such as a knife, causing direct trauma to brain tissue [2,16]. Besides velocity, the injury caliber is also determined by the intracranial path and energy/speed of object entry [3,14]. pTBI that contacts across the midline, passes through ventricles, or comes to rest in the posterior fossa, holds higher mortality rates in addition to projectiles maintaining higher velocity [3,14]. Consequently, the projectiles also determines the primary and secondary injury's austerity. Physics aside, other external factors determine the injury's severity. For instance, old age typically leads to a worse prognosis along with the pTBIs resulting from suicide attempts (due to closer proximity) [3].

III. PRIMARY AND SECONDARY INJURIES RELATED TO PTBI

Common primary injuries of pTBI include hemorrhage (blood loss), hematomas (blood clotted tissue), and parenchymal contusions (bruise of brain tissue) [1-3]. The risk of local wound infections, meningitis, ventriculitis, or cerebral abscess is also particularly high in pTBI patients due to contaminated foreign objects, skin, hair, and bone fragments along the projectile track [3-14]. But, one particularly evident resulting injury would be a porencephalic cyst (PC). When an object permeates the brain, a cavity typically results within the cerebral hemisphere. A PC is common in pTBI patients due to inflammation from the limited pool of hNSCs.[2] If CSF fills the cavity and affects the brain's communication with the ventricular system, this indicates the presence of a PC and is followed by a diagnosis of porencephaly, which is verified by a computed tomography (CT) scan [17]. Symptoms of a PC include visual field defects and brain behavior mimicking the presence of a stroke or brain tumor.

IV. CURRENT TREATMENT

A patient with pTBI is managed by a medical team that takes note of key information such as duration of loss of consciousness, seizures at any point in time, comorbidity (meaning the simultaneous presence of two diseases or conditions in a patient), and if any anticoagulants or antiplatelets (substances used to prevent and treat blood clots) were used [14].This early activation from the trauma team may aid in providing recognition of polytrauma (severely injured patients usually with two or more severe injuries in at least two areas of the body and an accurate severity assessment, considering the entry and exit points of the injury [3,14]. Primary analysis utilizing various neuroimaging techniques aid professionals in the evaluation and prognosis of pTBI. The Glasgow coma scale (GCS) is commonly taken of pTBI patients to scan for intracranial pressure [3,14]. A CT scan is also taken of a pTBI patient to evaluate the mass lesion or cerebral edema along with identifying the extent of any intracranial injury [3,14]. As this brief explanation of the process is undertaken in the current treatment indicates, there is a strong emphasis on resolving secondary injuries and symptoms rather than neuronal loss or axonal damage.

V. STEM CELLS

Stem cells (SC) are unspecialized, pluripotent cells, or cells that have the ability to give rise to any other type of cell within the body [7,8,18]. SCs differ from mature cells as mature cells are specialized and maintain a set function while SC's pluripotency makes them unique [14]. In order to be characterized as a stem cell, the cell must be able to self-renew (produce new stem cells) and differentiate (specialize into a specific cell type) [18]. Different classifications of stem cells affect these properties, as some stem cells might be multipotent and only give rise to cells in a specific family such as blood cells or totipotent and can form all cell types [8,11].

VI. EMBRYONIC STEM CELLS

Embryonic stem cells (ESC), while they are justifiable for TBI treatment as they maintain established protocols for maintenance in culture and are pluripotent (or able to give rise to any type of cell), are not researched thoroughly in current animal models of pTBI compared to hNSC [20]. Furthermore, generation of ESC is insufficient, unsure whether they would be rejected if used in transplants [20]. Therapies that use ESC lack concrete results and if derived directly from ESC undifferentiated culture prep can cause tumors and promote cancer development [20]. Applying hNSCs would avoid potential ethical issues associated with cell harvesting along with their multipotency with respect to differentiation into multiple neural phenotypes.

Author	Experimental Model	Significant Results
Hu et al.	Perilesional vs Intralesional transplant of hNSC in pTBI.	Perilesional mitigated secondary injury and reduced lesion size significantly compared to intralesional. Perilesional group also had signifigantly more motor cortex sparing and not persistance of a porcenphalic cyst, as evident in intralesional group.
Spurlock . M et al	Neuronal differentiation of hNSC post PBBI.	hNSC survived for at least 5 months. 150%of cells successfullt engrafted with 57% differentiation. Presence of presynaptic structural protein indicates integration with existing neural network.
Blaya M. et al	Genetically engineered MNS1 in hNSC transplantation post TBI.	Modified hNSC exhibited elevated survival rates. GFP/NeuN-positive cells doubled in engraftment in TBI MNS1 group compared to sham control. TBI MNS1 group had significantly greater hippo-campal neurogenesis and improved hippocampal dependent spatial memory capacity.
Elias P et al.	Implantation of Collagen scaffold seeded with hippocamapl models with pTBI.	Engraftment was low since scaffold didn't fill cavity. 1-2% cells remained in scaffold and BrdU survival was low. Positive astrocytes were detected around glial scarring.
Ruppert K et al.	CCI injured rats with transplanted MSC.	Significant increase of neurological deficits in early treatment. Delayed treatment demonstrated significant decrease in cognitive and neurological deficits as well as support for anti-inflammatory pathways.
Haus D et al.	hNSC transplant into immunodeficient rats with TBI.	hNSC groups preformed significantly greater in MWM test compared to sham. Demonstrated ameliorated impairment with improvement in learning locations. Improved host hippocampal neuron survival without harming lesion or host neuronal network while exhibiting migration throughout the host brain, extending in ventral hippocampal and ventral/medial cortical regions.

Table 1. Experimental models of stem cell transplants in TBI.

Differentiation and function improvement have been present in hNSCs experimental models already. At the cellular level, hNSC were also able to differentiate into neuronal and glial lineages, mitigate axonal damage, recapitulate host neural pathways, improve host neuron activity patterns and migrate beyond the lesion location (see Table 1) [5,10,12,20,21].Within the proliferation induced by hNSC transplant, the expression of nestin (cytoskeletal intermediate filament initially characterized in neural stem cells) was high post pTBI in the animal hNSCs transplant models [5,11,16,21]. Aside from differentiation, at the cellular level, hNSCs have restored expression of plasticity related Arc in host tissue, which has a key role in synaptic plasticity and memory consolidation. These results indicate that hNSCs transplantation in pTBI had rebuilt neuronal function [16].. However, it is important to keep in mind that it takes 1-3 months for hNSCs to fully differentiate into neurons, which explains why in most studies, after one week, the implanted neurons remained rounded and undifferentiated [11].

VII. MESENCHYMAL STEM CELLS (MSC)

While they are multipotent and have easier accessibility (because they can be isolated from various tissues), MSC treatment is not aimed to replace lost neurons, which is the main objective in any CNS stem cell transplant [13,19].An existing study incorporating MSC confirms these concerns as the study's inconsistency with cell quantity injected revealed no long-term engraftment and survival issues [13,20].

hNSC engraftment has been demonstrated extensively in experimental models. For instance, hNSCs were able to achieve 90% engraftment while interacting with existing neural microenvironments and reduce astrological scarring (scars evident in brain tissue) [2,5,10,15,16,20]. Although, in certain cases, transplanted hNSCs did not have any significant effect on reducing axon damage, hNSCs presents the best possible option for pTBI treatment due to their verified engraftment, lesion size reduction, and improvement of cognitive and motor deficits in rat pTBI and TBI models (see Table 1) [2,5,9–13,15,2].

VIII. NEURAL STEM CELLS (NSCs)

NSCs influence neuroblast migration toward the injury site, number of residential neurons and glial cells, astrogliosis, and locomotor recovery [11]. . That being said, hNSCs perform better compared to NSC as rat NSC gave rise to 27% new neurons while hNSCs gave rise to 57%.[5] hNSCs have also illustrated extended migration and differentiation outside the damaged tissue in cortical areas, the blood brain barrier (BBB), into vascular and endothelial cells, the medial ipsilateral cortex, the contralateral corpus callousness, and surrounding brain tissue [2,5,10,12,20,21]. Engraftment of hNSC has also been recognized long term, surpassing 5 months at least post transplantation and differentiation into mature neurons, astrocytes, and oligodendrocytes [5,19,20].

IX. LOCATION OF STEM CELLS TRANSPLANTATION

Location of hNSC transplantation influences the engraftment rate, migration, and impact on spatial and physical improvement [11,21]. Post pTBI, the resulting cavity lacks structural support and promotes apoptosis and neuronal death rather then engraftment [6]. Minutes to a few months after the pTBI was formed, pro-inflammatory cytokines that mobilize immune and glial cells to the injury environment, causing edema, inflammation, and demyelination (damage to the myelin sheath that surrounds neuronal fibers) [11]. The natural microenvironment at the brain with a raw pTBI cavity is not suitable for optimal success of transplanted stem cells, hence why transplanting the cells around the lesion would produce greater recovery and has been proven to do so in existing rat pTBI models [11,20,2].

An intralesional transplantation refers to the hNSC being transplanted directly into the resulting injury or cavity. A perilesional transplantation indicates that the subject was inserted around the cavity [2]. Comparing the two hNSC methods within a Sprague Dawley rat model of pTBI, the results of lesion size and motor cortex sparing of the perilesional group were significantly greater compared to the intralesional (see Figure 2) [2]. The study's foot fault test measured physical and cognitive deficits post transplantation. While there was significant lesion reduction and cortex sparing between the two groups, the test revealed insignificant data between the two in engraftment and behavioral difference. This leaves a gap in reasoning since significant cortex sparing should evidently produce significant behavioral differences.[2] Nonetheless, the perilesional transplantation lead to greater tissue/cortex preservation and should continue being tested and evaluated moving forward [2,5,12].

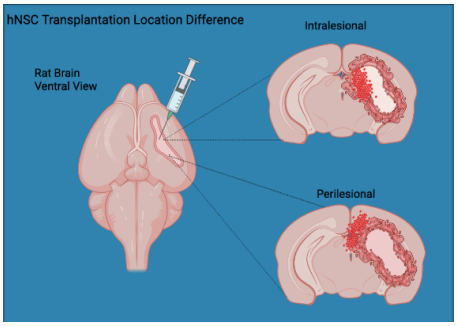

Figure 2. Perilesional transplantation compared to intralesional. Illustration of rat brain post experimental pTBI from the ventral view. Displaying the transplantation of hNSC, while demonstrating the difference between a perilesional and intralesional transplant in relation to the cavity (made using Biorender).

Another study confirmed the results previously presented as hNSCs reduced lesion size and increased neuronal differentiation through a perilesional transplantation.[5] Two main groups were observed: the sham or placebo group that had a mimicked pTBI but no cells transplanted (control group) and the transplant group that received the pTBI and cell transplantation. Even though latency was not significant between sham and transplant groups, this model conveyed the

perilesional transplant method reliable and viable, with 43% engraftment exhibited [5]. Previous animal models of transplanted hNSCs in other locations, such as contralaterally, or through a scaffold, left the cavity surrounded by glial scars, unfilled, and reduced migration (see Table 1) [10]. Present perilesional models have shown greater extent of differentiation and maturation [10].

X. FUTURE DIRECTIONS

Moving forward in hNSC model experimentation for pTBI treatments, a few factors should be considered. Primarily, a larger body of animal model experiments that evaluate engraftment and proliferation beyond two months should be conducted to work toward a potential human model. Along with engraftment, growth factors, and biogenic factors, promotional influences in differentiation should be considered further. Immunosuppression should also be acknowledged because of its critical nature in hNSC transplantation. As preclinical studies of TBI have generally established that hNSC transplantation was neuroprotective, the original lack of neuronal replacement is attributed to robust host immune system response rejection, which can be lessened through immunosuppression and promote greater engraftment in future studies.

XI. CONCLUSION

In summary, pTBI is a nationwide issue that could be ameliorated through hNSC in clinical practice. Current pTBI treatment, while good at managing secondary injury and symptoms, does not address the lost neurons nor the lesion head on. hNSC's potential to interact with host neural networks and restore neural connections effectively has been demonstrated through numerous animal models. By extending experiments past two months and observing long lasting activity of perilsionally transplanted hNSC, neurologists will be able to gauge how this treatment would function in human brains. Future studies should also focus on reducing the lesion along with behavioral and physical improvements, utilizing a wide variety of tests, for instance the Morris Water Maze test or the foot fault test to optimize progress. While further safety and mechanistic studies are warranted prior to the clinical trial phase, there is good evidence in support of a hNSC transplant as a treatment option for pTBI.

XII. REFRENCES

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