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# Amnion-derived stem cell transplantation: A novel treatment for neurological disorders

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## Abstract:

In this review, we evaluated the literature reporting the use of amniotic stem cells (ASCs) in regenerative medicine for the treatment of neurological disorders. There is an increasing amount of evidence that indicates the exacerbation of the primary injury by inflammation in neurological disorders characterized by rampant inflammation, thereby increasing damage to the central nervous system (CNS). To address this, we focus on the amnion cells' anti-inflammatory properties, which make their transplantation a promising treatment for these disorders. In addition, we offered insights into new applications of the ASC in the fields of regenerative medicine and tissue engineering.

## Key words:

Amniotic stem cells (ASCs), human amniotic epithelial cells (hAECs), human amniotic mesenchymal stromal cells (hAMSCs), inflammation, neurological disorders, stroke

## Amniotic Stem Cells as Transplantable Cells

The discovery of placental- and fetal membrane-derived stem cells has added a new venue in the field of cell therapy. Among the placental origin cells, one of the most interesting has been the amnion, which is the two-layered membrane (made up of an epithelial monolayer and a stromal layer) that lines the fluid-filled sac surrounding the fetus.<sup>[1]</sup> After birth, the amnion is expelled, along with the placenta, preventing any ethical concern about the use of amniotic stem cells (ASCs). The amnion has been found to contain two types of cells: membrane-derived stem cells and fluid-derived stem cells. Amniotic membrane-derived stem cells also subdivide into human amniotic mesenchymal stromal cells (hAMSCs) and human amniotic epithelial cells (hAECs).<sup>[2]</sup> The hAECs originate from the embryonic ectoderm and express embryonic and pluripotent stem cell markers.<sup>[2]</sup> Meanwhile, hAMSCs present phenotypic characteristics similar to those of bone marrow-derived mesenchymal cells, and are harvested from the extraembryonic mesoderm.<sup>[2-5]</sup> There are several published works about neuroglial differentiation of hAECs. In one

article, the ability of hAECs to exert neural-like activity and their multipotentiality to become neurons, astrocytes, or oligodendrocytes was demonstrated.<sup>[6]</sup> A recent study in peripheral nerve injury differentiated hAECs into Schwann cells in order to help nerve regeneration and function. In this experiment, brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) concentrations were upregulated, suggesting that they may play a key role in neuroregeneration.<sup>[7]</sup> Likewise, the ability of hAMSCs to proliferate as neural progenitor cells and their further potency on becoming glial cells has been demonstrated.<sup>[8]</sup>

In the studies of ASC transplants, neither allogeneic nor xenogeneic transplants have been shown to induce an immune response, making their therapeutic use a viable, low-risk treatment option.<sup>[9,10]</sup> Human ASC shows low immunogenicity due to their expression of surface characteristics of antigen-presenting cells such as human leukocyte antigens (HLAs), specifically HLA-G, which has an important role in peripheral immune tolerance during pregnancy.<sup>[11]</sup> The expression of anti-inflammatory proteins interleukin (IL)-10, IL-1 receptor agonist, and tissue inhibitors of metalloproteinases 1-4 by hAEC and hAMSC further expands the potential therapeutic

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field of these cells to include autoimmune and degenerative disorders.<sup>[12]</sup> Furthermore, studies with human amnion cell transplantation into animal models have shown no induction of oncogenesis.<sup>[11]</sup> A study evaluating tumorigenicity by karyotype analysis has demonstrated that because of their chromosomal stability, ASCs represent a low-risk treatment option.<sup>[13]</sup> The lack of ethical dilemmas, the capacity to differentiate into a variety of tissues, the absence of an immune response, and the secretion of anti-inflammatory proteins by ASC makes them a perfect candidate for use in regenerative medicine.

### Therapeutic Applications of Amniotic Stem Cells

Recent studies have found a wide range of therapeutic applications for human amniotic membrane and amniotic fluid stem cells. Among these are the promotion of reepithelization, the modulation of cell differentiation and formation of new vessels, and a reduction in fibrosis, apoptosis, and inflammation.<sup>[14-17]</sup> Currently, amnion-derived tissues have been obtained with the objective of using them in the clinical field. These are widely used for skin burns and wound healing in traumatology and general surgery.<sup>[18-20]</sup> These tissues have also been transplanted for the treatment of conditions such as diabetic foot ulcers and venous leg ulcers.<sup>[21-23]</sup> Altogether, these results suggest that ASCs could provide beneficial outcomes for several conditions. Nevertheless, further preclinical research is needed to standardize their isolation and differentiation, with the objective of expanding their use to other medical areas.

#### Amnion cells in neurological disorders

Studies evaluating the effect of ASCs in new therapies have been geared toward their utility in several neurological disorders including Parkinson's disease (PD), stroke, traumatic brain injury (TBI), and spinal cord injury (SCI).<sup>[1]</sup> Reports show that hAECs may be able to differentiate into different cells of the nervous system such as astrocytes, oligodendrocytes, and neurons.<sup>[6]</sup> In addition, these stem cells are capable of creating and secreting neurotrophic factors and neurotransmitters.<sup>[16,17]</sup> These characteristics confer them neuroprotective and neuroregenerative properties, which have been observed in the early stages of injury in preclinical studies.<sup>[16,17]</sup>

The capacity to differentiate into different neural cells greatly expands the viability of hAECs as a treatment for stroke.<sup>[1,10,24]</sup> In one study, rats underwent a middle cerebral artery occlusion in order to produce a stroke model and were treated with an hAEC injection into the dorsolateral striatum on the first day after stroke. Here, the hAECs were shown to be capable of differentiation into astrocyte- and neuron-like cells.<sup>[17]</sup> In another experiment, hAECs were transplanted into a hemorrhagic stroke model in rats, improving motor skills, and reducing cerebral edema, with survival of transplanted cells in the lateral ventricular wall at 4 weeks.<sup>[25]</sup> The efficacy of hAEC transplantation in PD has also been evaluated in several studies. One of these evaluated a rat model of PD with 6-hydroxydopamine lesions, observing a higher survival rate of cells in dopaminergic neurons, and showcasing the protective effects of hAEC transplantation.<sup>[26]</sup> An increase in the quantity of dopaminergic cells in the substantia nigra and no overgrowth of the stem cells were observed after grafting, leading to the conclusion that hAEC transplantation may

be a viable treatment for PD, counteracting the depletion of dopaminergic neurons.<sup>[26]</sup>

Several studies have expanded on the use of ASC therapy in other diseases as well. In one study, a rat TBI model was treated with an hAMSC transplant, leading to improved brain function, brain tissue morphology, and increased quantities of nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3), ciliary neurotrophic factor, and GDNF.<sup>[27]</sup> Further studies on TBI used amnion-derived multipotent progenitor stem cells to reduce the resulting decay of axons in the thalamus and the *corpus callosum*.<sup>[28]</sup> Another interesting field of research for the use of ASC transplantation has been SCI. In a contusion model of SCI in monkeys, ASCs were transplanted into the injury, resulting in an increase of new host axons and reduced degeneration of axotomized spinal cord neurons.<sup>[29]</sup>

#### Use of amniotic stem cells in noncentral nervous system-related diseases

Although the focus of this review is mainly the application of hAEC/hAMSC in primarily neurological disorders, a wide variety of disorders (including but not limited to cardiovascular,<sup>[30]</sup> pulmonary,<sup>[31]</sup> hepatic,<sup>[32]</sup> pancreatic,<sup>[33]</sup> muscular,<sup>[34]</sup> and cartilage<sup>[35]</sup>) may affect the CNS as well.

Amniotic membrane stem cells have a variety of effects outside the nervous system. Among their effects on the cardiovascular system, the production of cardioprotective factors stands out. In a rat heart ischemia model, human amniotic membrane cells were transplanted in order to observe their ability to inhibit injury to the myocardium after ischemia and cardiac dysfunction. After the transplant, rats exhibited an improvement in cardiac contractile function and ejection fraction.<sup>[36]</sup> Another instance exhibited a reduction of myocardial scarring and inhibited thinning in myocardial infarction after a stem cell transplant.<sup>[37]</sup> Nevertheless, other studies have been unable to prove the differentiation of amniotic membrane stem cells toward cardiomyocytes, continuing the debate on the subject.<sup>[38]</sup>

The capacity of hAECs to differentiate into type II pneumocytes has also been explored in several studies. One of the studies that evaluate the effect of hAEC in lung injury induced lung inflammation and fibrosis in a mouse model with bleomycin, treating the mice with an hAEC transplant.<sup>[39]</sup> After the transplant, hAECs were able to differentiate into phenotypic alveolar epithelium and secrete surfactant protein.<sup>[39]</sup> The application of hAEC also helped fight lung fibrosis, with a reduction of lung collagen, and inflammatory and fibrotic cytokines.<sup>[39]</sup> A similar study examined the effect of fetal membrane-derived cells on bleomycin-induced lung fibrosis in a mouse model.<sup>[40]</sup> ASCs were administered by several routes, depending on their origin: Xenogeneic and allogeneic cells were transplanted through either intraperitoneal or intratracheal, and allogeneic cells through the intravenous route of administration.<sup>[40]</sup> The results showed a reduction in neutrophil infiltration and the severity of lung fibrosis significantly decreased in the stem cell-treated group.<sup>[40]</sup>

The potential of human amniotic membrane-derived stem cells for the treatment of hepatic disorders has also been examined. The capacity of these cells to differentiate into liver cells was

evaluated, using periodic acid-Schiff staining on amniotic membrane cells in order to evaluate glycogen storage.<sup>[41]</sup> The positive staining demonstrated that the amniotic membrane cells were able to carry on important physiological function of hepatocytes such as glycogen storage.<sup>[41]</sup> In a similar study, rat amniotic membrane cells were found to be capable of low-density lipoprotein (LDL) uptake after being exposed to an environment suitable for hepatic differentiation.<sup>[42]</sup> Another disorder of the liver in which the use of amnion cells was found to be useful was biliary fibrosis, characterized by the loss of hepatic function due to fibrotic remodeling. The only treatment available at the moment is liver transplant but a study on a rat model of biliary fibrosis showed that treatment with human ASCs may be a viable option. In this study, a patch made of human amniotic membrane was placed on the liver surface after biliary duct ligation.<sup>[43]</sup> After treatment, the severity of fibrosis was reduced, and no signs of cirrhosis were present. These observations suggest that amniotic membrane patches applied directly onto the liver may protect against damage from fibrosis.<sup>[43]</sup>

### Role Of Amniotic Stem Cells in Modulating Inflammation

In the early stages of neurological disorders, both the short- and long-term responses mounted by brain cells can have a detrimental effect. The primary injury results from the initial trauma, and consists of the loss of neurons and necrosis.<sup>[44]</sup> At this stage, a biochemical cascade begins, eventually leading to degeneration and secondary cell death.<sup>[45]</sup> The progressive nature of the injury has been shown to produce a chronic inflammatory state, worsening cell death, and therefore, neurological damage.<sup>[44]</sup>

During the acute phase of primary injury, such as stroke or TBI, inflammation provides a protective environment for damaged cells, acting as a defense mechanism against infectious microorganisms. However, inflammation can be detrimental to cell recovery in the chronic phase, worsening the preexistent damage from the primary injury.<sup>[46]</sup> The initial release of necrotic cell products, such as ATP, UTP, and high-mobility group protein B1 serve as chemotactic factors, stimulating the migration of immune cells into the CNS.<sup>[24]</sup> These cells include monocytes, macrophages, dendritic cells, T cells, neutrophils, and B cells, which permeate through the blood-brain barrier (BBB) in order to clear dead tissue and promote neuroregeneration.<sup>[24]</sup> The production of proinflammatory cytokines and molecules, such as interferon- $\gamma$ , reactive oxygen species (ROS), IL-6 and IL-17, nitric oxide (NO), matrix metalloproteinase, and tumor necrosis factor (TNF) by these cells, lead to an increase of immune cell and glia migration toward the intrathecal compartment.<sup>[24,47]</sup> This, in turn, creates an adverse environment for regeneration, with an increased immune response leading to an aggravation of neural damage during the chronic stage.

One of the most interesting characteristics of amnion cells is their ability to modulate the immune response by inhibiting excessive inflammation.<sup>[47]</sup> Amnion cells have been shown to possess the ability both to suppress the proinflammatory cytokines produced by the immune cells at the injury site, and produce anti-inflammatory cytokines such as IL-10 and

IL-6.<sup>[48]</sup> Among the various immunomodulating effects of ASCs are the inhibition of metalloproteinases, reduction of antigen-presenting cells, upregulation of heat shock protein 27 (hsp27), suppression of T cell multiplication, and modification of M1 microglia, which favor inflammation, into the anti-inflammatory M2 type.<sup>[49-51]</sup> This reversal from an environment favorable for inflammation into an anti-inflammatory one promotes the regeneration of damaged brain cells.<sup>[24]</sup>

Although inflammation initially has a beneficial effect on stroke and TBI, its prolonged presence can be detrimental. In order to provide the best treatment, the application of ASC has to be administered at a specific time window, exploiting the benefits of both inflammation and the cell's anti-inflammatory properties. If given too early, ASCs may worsen the inflammatory process in its benefic stage, suppressing chemokines such as stromal cell-derived factor-1 (SDF-1). This chemokine helps transplanted cells migrate toward the site of injury so that its suppression would hinder the transplanted cells' ability to reach the injured area.<sup>[24]</sup> Therefore, ASC therapy must be regulated in order to favor inflammation in the early stages of brain injury and to suppress it in the later stages.

### Amnion Cell Therapy in Inflammation-Plagued Stroke

Among the most common causes of death in the United States, stroke ranks fourth, with ischemic stroke being the most common variety.<sup>[51]</sup> Currently, ischemic stroke is treated with tissue plasminogen activator (tPA) and therapies derived from it.<sup>[52]</sup> However, this treatment carries a risk of severe complications and a small therapeutic window, along with a series of contraindications (such as blood glucose level, blood pressure, and age).<sup>[53-55]</sup> Evidence shows that inflammation in stroke is not only caused by the primary injury but can also occur later as a result of the events occurring after stroke.<sup>[54,55]</sup> The area around the lesion, known as the ischemic penumbra, suffers from low oxygen levels due to the cutoff in blood supply. This lack of oxygen causes astrocytes and microglia to produce proinflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-4, and chemokines such as NO.<sup>[54,56]</sup> As previously mentioned, inflammation plays an important protective role in the early phase of stroke but increases the damage done to the brain when prolonged. Therefore, astrocytes and microglia not only have the capacity to secrete proinflammatory substances but can also produce anti-inflammatory factors (such as BDNF, erythropoietin, vascular endothelial growth factor, and others). Nevertheless, these regulatory mechanisms often fall short of preventing further damage and recovering neurological function, creating the need for an appropriate therapy.

Since inflammation is a process that takes place over an extended period of time, there are a variety of possible treatment options. Evidence from animal models of stroke has found treatment with stem cells to be a safe and effective alternative. Among the available stem cell therapies, hAMSCs have shown promising results.<sup>[57]</sup> Human placenta stem cells, as described above, have a great potential for differentiation.<sup>[58]</sup> Due to their inflammation-modulating capacity, treatment with these cells allows us to fully take advantage of the beneficial effects of inflammation in the early stages while regulating it later on.<sup>[59-61]</sup> Therefore, the best time to inject ASCs is during the early stages of stroke.

As stated previously, these stem cells have the ability to reduce proinflammatory signals and secrete anti-inflammatory and neurotrophic factors, promoting healing and inhibiting inflammation. In animal models, placenta-derived stem cell transplants have been observed to lead to a better outcome though their exact mechanism in stroke treatment is not yet completely understood. Nevertheless, most studies have shown evidence pointing toward the stem cells' ability to regulate inflammation as the cornerstone of this therapy.

In order to regulate inflammation, the recognition of the role of stem cells in various key points of inflammation signaling cascades is warranted. In an *in vitro* stroke model, hAECs were shown to confer neuroprotection by acting on melatonin receptor type 1A (MT1) and the cells' ability to survive increased when administered with a simultaneous melatonin treatment.<sup>[62]</sup> Another study of *in vivo* and *in vitro* stroke models found that dog placenta cells (DPC) had neuroprotective properties by way of Hsp27.<sup>[49]</sup> In this study, DPCs were shown to increase the expression of Hsp27 near the area surrounding the ischemic lesion.<sup>[45]</sup> Since Hsp27 has been shown to have neuroprotective effects, its increased expression is indicative of the mechanism through which DPCs confer neuroprotection.<sup>[63-65]</sup> These studies evaluated the different stages of inflammatory mechanisms, demonstrating that ASCs could have neuroprotective capacities through immunomodulation.

### Amniotic Stem Cell Applications in Regenerative Medicine: Possible Delivery Mechanisms

One of the possible applications of ASCs in the treatment of stroke and TBI is through a subdural patch. This method consists of placing a patch of amniotic membrane directly on the brain, harnessing its therapeutic properties, and providing a nonimmunogenic graft due to its ability to differentiate and integrate into brain tissue.<sup>[66]</sup> The stem cells from the patch would facilitate the movement of pluripotent cells, both transplanted and generated in the brain, toward the injury site.<sup>[67]</sup> These connections, or biobridges, have been observed in previous studies utilizing human bone marrow-derived mesenchymal stromal cells as treatment for TBI in a rat model.<sup>[67]</sup> The ability to form biobridges and differentiate into neural progenitor cells makes amniotic subdural patches an ideal therapy not just for regulating inflammation but for promoting neuroregeneration as well. Although there have not been any experimental models of biobridge formation in stroke, it has been hypothesized that stem cells have the capacity to act in a similar manner, allowing neurogenic stem cells to migrate toward the stroke core and ischemic penumbra.<sup>[68]</sup>

The ability to regulate inflammation makes hAECs useful in the treatment of inflammatory neural disorders.<sup>[24]</sup> The immunomodulatory factors secreted by hAEC, such as prostaglandin E-2 (PGE2), regulate the immune response, suppressing inflammation and thus reducing its adverse effects.<sup>[69]</sup> Another interesting characteristic of these cells is their expression of human leukocyte antigen G (HLA-G), which allows them to evade the immune system and induce apoptosis in immune cells.<sup>[24]</sup> This effect has also been observed in multiple sclerosis (MS) mouse models where alpha-fetoprotein inhibits inflammation, and outside the CNS in lung injury models in which hAEC cells inhibited the secretion of proinflammatory

cytokines and increased secretion of anti-inflammatory cytokines.<sup>[24]</sup> Modifying hAECs in order to produce anti-inflammatory cytokines and chemokines *in vivo* would permit the quantification and evaluation of their clinical use in diseases associated with inflammation. In addition, the use of these factors alongside ASC therapy would potentiate their anti-inflammatory effects, providing a more effective treatment.

Both hAECs and amniotic membrane grafts have similar immunomodulatory properties, which have been described above. Amniotic membrane patches have been shown to promote the formation of a microenvironment conducive to injury-healing by host cells, allowing for better wound-healing.<sup>[30,34,37]</sup> Another advantage of human amniotic membrane patches is their relative ease of preparation, requiring only the separation of the amnion from the chorion without the need for culture or isolation.<sup>[30]</sup> However, their therapeutic action stems from the modulation of the injury microenvironment, rather than differentiation of ASCs. On the other hand, hAECs have exhibited the ability to differentiate into neural stem cells as well as modulate the inflammatory response, increasing their potential field of action.<sup>[1,6,10,24]</sup> Despite the beneficial effects shown by undifferentiated hAEC transplantation, trials conducted with neural stem-like cells acquired from differentiated hAEC have shown an increased secretion of neurotrophic factors.<sup>[28]</sup> Therefore, hAEC should be cultured in a medium conducive to differentiation in order to fully harness their therapeutic potential.

The transplantation of tissues from one species to another, known as xenografting, has shown a lot of promise in the treatment of a variety of diseases. However, a variety of immunologic complications arise due to rejection of the grafts by the host's immune system. This rejection leads to the production of xenoreactive antibodies, which cause systemic inflammation and activate the complement system.<sup>[70,71]</sup> Though a variety of tolerance techniques have been tried, such as neonatal desensitization, these have not improved the rate of rejection.<sup>[72]</sup> An option that has worked in previous studies has been the application of circulating anti-inflammatory alpha-1-antitrypsin (ATT), along with anti-CD4/CD8 therapy, protecting xenografts from inflammation and therefore, from rejection.<sup>[73]</sup> This mechanism opens up the possibility of using hASCs to prevent xenograft rejection by modulating the immune response.<sup>[73]</sup> By transplanting hASC at the same time as xenografts, their rate of rejection would decrease.

Allografts, or the transplantation of tissue from one individual to another of the same species, are not immune to rejection, with the possibility of being targeted by the host's defenses being very high. The first obstacle encountered by allografts is the host's innate immune response, which is nonspecific. The allograft then faces the specific immune response, mediated by T cells specifically designed to recognize alloantigens.<sup>[74]</sup> A number of studies have utilized ASCs in conjunction with allografts in order to modulate the immune response to the graft, reduce the necessity for immunosuppressive treatment, and favor acceptance by the host.<sup>[75]</sup>

The inflammation that characterizes CNS disorders is not only present in the CNS but also reaches the peripheral nervous system (PNS).<sup>[76-79]</sup> This involvement of the PNS in

inflammatory response after injury creates an interesting array of diagnostic and therapeutical opportunities such as the possibility of treating PNS inflammation with ASCs. Among the peripheral organs that present inflammation after stroke is the spleen, which could easily be targeted by stem cells.<sup>[80,81]</sup> This would be accomplished either by a systemic transplantation of stem cells directed specifically to the spleen or by transplanting the cells into the organ. Once ASCs reach the spleen, their anti-inflammatory properties alleviate peripheral inflammation.

Although there are many pluripotent cells derived from gestational tissues (such as umbilical cord blood, Wharton's jelly, and amniotic fluid), ASCs are the only ones that can be used without previous cultivation.<sup>[82]</sup> Since hAECs have shown a very diverse potential for differentiation *in vitro*, they could be useful in a variety of diseases. Studies have demonstrated their ability to differentiate into a number of tissues including adipose, cardiomyocytic, pancreatic, hepatic, neural, osteogenic, and chondrogenic.<sup>[83-85]</sup> Therefore, ASCs could be harvested at birth and directed toward specific treatments for those at risk of developing diseases. Through genetic testing, individuals at risk of developing diseases in their adulthood could start with prophylactic ASC therapy, slowing down or even stopping the development of these disorders.

### Future Applications of Amniotic Stem Cell Therapy in Central Nervous System Diseases and Inflammation

Throughout the text, substantial evidence of the therapeutic potential of ASCs has been presented. Among these experimental applications, their use in regenerative medicine for neurological diseases stands out, as well as in other pathologies that affect the nervous system such as cardiovascular, respiratory, hepatic, pancreatic, and muscular disorders. Despite the amount of evidence of amnion cell therapy's efficacy in the treatment of CNS and non-CNS disorders in animal models, their application still needs to be refined and adapted for clinical application. Among the many translational issues, an effective and less invasive application route for stem cells needs to be determined as well as the need for optimization of doses and timing of their delivery in clinically relevant animal models. The regulatory effects of ASCs on inflammation must be taken into account in order to determine the safe and effective therapy for different diseases. Consequently, more research is still needed in order to determine and standardize the optimal stem cell transplantation treatment in diseases characterized by inflammation.

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#### Conflicts of interest

There are no conflicts of interest.

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