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Amnion: A Potent Graft Source for Cell Therapy in Stroke

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Regenerative medicine is a new field primarily based on the concept of transplanting exogenous or stimulating endogenous stem cells to generate biological substitutes and improve tissue functions. Recently, amnion-derived cells have been reported to have multipotent differentiation ability, and these cells have attracted attention as a novel cell source for cell transplantation therapy. Cells isolated from amniotic membrane can differentiate into all three germ layers, have low immunogenicity and anti-inflammatory function, and do not require the destruction of human embryos for their isolation, thus circumventing the ethical debate commonly associated with the use of human embryonic stem cells. Accumulating evidence now suggests that the amnion, which had been discarded after parturition, is a highly potent transplant material in the field of regenerative medicine. In this report, we review the current progress on the characterization of MSCs derived from the amnion as a remarkable transplantable cell population with therapeutic potential for multiple CNS disorders, especially stroke.

Key words: Adult stem cells; Progenitors; Placenta; Mesenchymal stromal cells; Transplantation; Cerebral ischemia

Ischemic stroke is a leading cause of death and long-lasting disability. Ischemic stroke results from a transient or permanent reduction in cerebral blood flow that is restricted to the territory of a major brain artery (11, 12). The reduction in flow is, in most cases, caused by the occlusion of a cerebral artery either by an embolus or by local thrombosis. Transient focal cerebral ischemia initiates a cascade of detrimental events including accumulation of intracellular Ca\(^{2+}\) and formation of free radicals. When blood flow is restored, oxygen can enhance the biochemical reactions that generate free radicals, which can damage the lipid constituents of cellular and organelle membranes leading to neuronal death (40). This cell death can be divided into two broad categories: early necrotic death of cells in the ischemic core and delayed death of susceptible neurons in other neighboring regions, the so-called penumbra. Because the secondary cellular death occurs over an extended time, these neurons have the potential to be rescued by pharmacologic agents (37,39). More importantly, the recently recognized delay in stroke-induced pathophysiologic alterations has prompted investigations on neurorestorative treatments, including cell therapy, to salvage the ischemic penumbra and promote functional recovery from stroke. Neurorestoration, thus significantly extends the therapeutic window, which to date is limited to the 3-h window rendered by the thrombolytic agent tissue plasminogen activator and only benefits about 3% of the ischemic stroke population. If proven effective, such cell therapy will greatly impact on the treatment and management of stroke.

ADULT STEM CELL

Regenerative medicine exploits the potential of transplanting exogenous or stimulating endogenous stem cells...
as a neurorestorative mechanism in ameliorating stroke deficits. Stem cells are undifferentiated cells capable of indefinite self-renewal and differentiation into specific cell lineages, conceivably posing as the universal donor cell in cellular replacement therapies in the treatment of a large number of genetic and degenerative human diseases, including hematopoietic and immune system disorders, diabetes, heart failures, chronic liver injuries, and neurodegenerative disorders. According to the developmental stage from which they are obtained, stem cells are generally classified into embryonic, fetal, or adult stem cells. Embryonic stem (ES) cells are pluripotent and can give rise to all specialized cell types of the organism. However, numerous considerations, both ethical and technical, limit the availability of these cells, which can only be isolated from the inner cell mass of early embryos. Moreover, the tumorigenicity of ES cells has yet to be resolved. In contrast, adult stem cells are rare cells thought to be present in all tissues and responsible for maintaining the homeostasis of the specific tissue. Adult stem cells (ASCs), previously thought to be limited in potential, have increasingly been shown to be able to differentiate into tissues of an entirely different germ layer. One of the most extensively studied populations of multipotent ASCs has been mesenchymal stem cells (MSCs) from the bone marrow, which represent a rare population of multipotent progenitors capable of supporting hematopoiesis and differentiating into the three classical mesodermal lineages (osteogenic, adipogenic, and chondrogenic). In vitro differentiation toward myogenic, neurogenic, endothelial, and hematopoietic pathway was also reported (4,9). MSCs have been also isolated from other tissues such as umbilical cord blood (16,17), synovium (10), periosteum (21), peripheral blood (81), adipose tissue (56,88), skeletal muscle (5), and, more recently, from placental tissue (54).

AMNION-DERIVED CELLS

Placenta-derived stem cells draw great interest as potential cells for regenerative medicine: their plasticity, the low immunogenicity, and the lack of ethical barriers to their procurement make them ideal candidates for restoring tissue in disease treatment. Among placental tissue multipotent cells have been isolated from the amniotic and chorionic membranes (2,31,45,55,71), from the umbilical cord (46), and from the trophoblast (20). In particular, amniotic membrane has been applied clinically (Table 1), and more recently, the cells from this membrane have been largely investigated (54).

The amnion is a thin avascular tissue, which represents the innermost layer of the sac that encloses the fetus. Amnion is comprised of two layers: an epithelial monolayer and a stromal layer. No invasive procedure is necessary to obtain the amnion because the placenta is expelled naturally after the delivery. From human amnion it is possible to isolate principally two cell types, which have been definitely defined in a recent report as human amniotic epithelial cells (hAEC) and human amniotic mesenchymal stromal cells (hAMSC) (54). These two cell types have a different embryological origin; whereas hAEC are derived from the embryonic ectoderm, hAMSC originate from the extraembryonic mesoderm (53,54).

Cells from the hAEC have been shown to express embryonic and pluripotent stem cell markers (45), while hAMSC display a phenotype comparable to mesenchymal cells isolated from bone marrow (31,55,71). Both hAEC and hAMSC have been shown to differentiate in vitro toward the classical mesodermal lineages (osteogenic, chondrogenic, adipogenic), but differentiation toward endoderm and ectoderm was also reported (54). With respect to the present application the hAEC differentiation toward neuronal lineage is widely described by the group of Sakuragawa and coworkers, who demonstrated that hAEC express markers of glial and neuronal progenitor cells (60), and have multiple neuronal functions such as synthesis and release of acetylcholine, catecholamines, neurotrophic factors, activin, and noggin (15,59,80). Neuronal and glial marker expression, as well as in vitro differentiation into neuroglial phenotypes, was also reported for hAMSC (58).

In perinatal medicine, MSCs from the placenta could be clinically used as autologous grafts for fetuses and newborns in peripartum tissue regeneration or for in utero transplantation in case of genetic disorders without immunologic rejection by the recipient (36,55). Furthermore, compared with allogeneic embryonic stem cells, ethical concerns and technical issues regarding graft rejection are not inherent in amnion-derived cells. Cells isolated from amniotic membrane do not elicit an allogeneic or xenogeneic immune response, but actively suppress lymphocyte proliferation (2,83). Additionally, long-term engraftment was observed after intraperitoneal or intravenous injection of human amniochorionic cells into newborn swine and rats, with human microchimerism detected in different organs, suggesting active migration and integration into specific organs, and indicating active tolerance of the xenogeneic cells (2). It has recently been shown that amnion-derived cells have immunomodulatory properties containing subpopulations with either T-cell-suppressive or stimulatory capabilities (41).

Experimental and clinical studies have demonstrated that amniotic membrane transplantation promotes reepithelialization, decreases inflammation and fibrosis (70), and modulates angiogenesis (13). Several growth factors produced from amniotic membrane are involved in these
Table 1. Research Progress in Amniotic Membrane Grafts: 2005–2008

<table>
<thead>
<tr>
<th>Year</th>
<th>References</th>
<th>Publications</th>
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<tr>
<td>2005</td>
<td>77</td>
<td>Amniotic membrane graft: histopathological findings in five cases.</td>
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<td>Amniotic membrane transplantation in ocular surface disorders.</td>
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<td>Intraoperative mitomycin C and amniotic membrane transplantation for fornix reconstruction in severe cicatricial ocular surface diseases.</td>
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<td>Amniotic membrane transplantation for ocular surface reconstruction.</td>
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<td>Survival analysis of conjunctival limbal grafts and amniotic membrane transplantation in eyes with total limbal stem cell deficiency.</td>
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<td>Follow-up of patients with ocular scarring secondary to LOC syndrome treated by amniotic membrane transplantation.</td>
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<td>Adhesion complex in cultivated limbal epithelium on amniotic membrane after in vivo transplantation.</td>
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<td>24</td>
<td>Limbal graft and/or amniotic membrane transplantation in the treatment of ocular burns.</td>
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<td>2006</td>
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<td>Nonpreserved human amniotic membrane transplantation for conjunctival reconstruction after excision of extensive ocular surface neoplasia.</td>
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<td>52</td>
<td>Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell.</td>
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<td>Technique of cultivating limbal derived corneal epithelium on human amniotic membrane for clinical transplantation.</td>
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<td>Advantages of amniotic membrane transplantation in eye surface diseases.</td>
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<td>Transplantation of human amniotic epithelial cells improves hindlimb function in rats with spinal cord injury.</td>
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<td>2007</td>
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<td>Fate of amnion-derived stem cells transplanted to the fetal rat brain: migration, survival and differentiation.</td>
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<td>44</td>
<td>Enhanced neural differentiation of neural stem cells and neurite growth by amniotic epithelial cell coculture.</td>
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<td>Role of amniotic membrane graft for ocular chemical and thermal injuries.</td>
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<td>Amniotic membrane transplantation for ocular disease: a review of the first 233 cases from the UK user group.</td>
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<td>Amniotic membrane transplantation: a review of current indications in the management of ophthalmic disorders.</td>
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<td></td>
<td>76</td>
<td>The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues.</td>
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<tr>
<td>2008</td>
<td>38</td>
<td>Human amniotic epithelial cells ameliorate behavioral dysfunction and reduce infarct size in the rat middle cerebral artery occlusion model.</td>
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<td>27</td>
<td>Sutureless amniotic membrane transplantation for partial limbal stem cell deficiency.</td>
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<tr>
<td></td>
<td>32</td>
<td>Surgery of the cornea: corneal, limbal stem cell, and amniotic membrane transplantation.</td>
</tr>
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processes, such as TGF-β, bFGF (68), EGF, TGF-α, keratinocyte growth factor, and hepatocyte growth factor (29).

**MSC APPLICATION IN HUMAN DISORDERS**

If MSCs derived from amnion recapitulate the MSCs harvested from bone marrow, their use as donor cell transplantation therapy in adult stroke is attractive. MSC therapy has already been used to treat patients with cancer. Moreover, data now demonstrate the potential of both autologous and allogeneic MSCs as transplantable cells, the latter allowing a mismatched donor–recipient regimen, with limited or even suppressed host immune reactions to the grafted cells (51). Preclinical studies have established the potential for MSCs to be a useful and safe treatment for stroke in humans. In parallel to adult stroke, early transplantation of bone marrow-derived multipotent adult progenitor cells (MAPCs) has been shown to ameliorate behavioral deficits induced by neonatal hypoxic-ischemic injury (86). Validations of efficacy and safety of MSC and MAPC are now under pivotal investigations and poised in the near future to reach limited clinical trials in adult stroke and neonatal hypoxic-ischemic injury. To date, we have seen only a few translation of cell therapy for stroke from the laboratory to the clinic (3,30,64). Caution must be exercised on the clinical applications of cell therapy for stroke and other human disorders until scientific evidence unequivocally
supports the therapeutic benefits and safety of such treatment.

Amnion-Derived Cells for Treating Brain Disorders

Preclinical studies in animal models have demonstrated that hAEC perform important hepatic, cardiomyogenic, and neural functions. In animal models of central nervous system disorders, hAEC exert neuroprotection in acute phases of injury and facilitating neuroregeneration.

Conditioned medium collected from hAEC has been shown to be neurotrophic for rat cortical cells (80) and supported the survival of chicken neural retinal cells (74). Transplantation of hAEC in a rat model of Parkinson’s disease produced dopamine and prevented neurons degeneration (25,26). After transplantation of hAEC into the lesioned areas of a contusion model of spinal cord injury in monkeys without immunosuppression, cells survived up to 120 days with no evidence of inflammation or rejection. Furthermore, improved performance in locomotor tests was observed in cell-treated animals compared to lesion control animals (62), suggesting neuroprotection and improvement of pyramidal and extrapyramidal systems of motor tracts controlling locomotion.

Cell Therapy for Stroke

MSC Transplantation. The preclinical validation of cell therapy for stroke should include optimization of cell delivery routes, dose, and timing. Proposed routes of cell administration include intra-arterial delivery, as in patients with myocardial infarcts (72,78), or intraleisonal implantation (34,35). However, logistical limitations may impede on the chosen injection path. For example, intra-arterial infusion of high doses of cells and angiography itself may cause adverse effects, including recurrent stroke (82). Similarly, surgical procedures in patients with severely disabling stroke are often impossible and exacerbate the patient’s state. The availability of a minimally invasive procedure for cell transplantation in stroke may prove advantageous. Of note, animal experiments have demonstrated that behavioral recovery after both intracarotid and intracerebral administration of bone marrow stromal cells was similar to that after intravenous administration (8,33,34), suggesting that the latter injection path, being much less invasive than the former routes, is an efficacious approach for cell delivery in stroke. Corollary to demonstrating the potential of peripheral administration is to determine the optimal dose for intravenous delivery that will provide efficacy that is safe and well tolerated by the transplant recipient.

To this end, laboratory studies have demonstrated dose-dependent effects of intravenous cell grafts in stroke animal models (73). After peripheral injection, MSCs cross the blood–brain barrier preferentially in areas that have experienced brain damage (8,14). Intravenous application of MSCs reduced apoptosis and promoted endogenous cellular proliferation after stroke (7). Stroke animals display behavioral and histological improvements with MSCs transplantation (8,33,84,87). Cell dose-dependent functional recovery accompanies such MSC transplantation in stroke animals (8), and the long-term effects of this approach are being recognized (67). Next, because the stroke occurrence is unpredictable, the optimal time at which cell infusion should occur after a stroke remains as another critical factor to ensuring the success and clinical feasibility of cell therapy for stroke. In animal studies, cells have been injected from 1 day to 1 month after MCA occlusion, and these investigations have examined whether transplantation at different times after ischemic damage affects proliferation, differentiation, integration, and functional outcome (51). Considerations, however, also need to focus on the response of the injured host brain to the surgical procedure and eventual lodging of the cells proximal to the ischemic tissues. Transplantation to an acute infarct would be unlikely to succeed if there were severe arterial occlusions, because blood flow would be inadequate to support donor cell viability. In addition, the release of excitotoxic neurotransmitters, free radicals, and proinflammatory mediators might threaten cells introduced into the peri-infarct region. The timing of transplantation also must consider the natural course of recovery from stroke. These multiple host microenvironmental influences on the grafted cells have prompted investigations into improving graft survival in concert with enhancing the viability of the ischemic host tissue via simultaneous injection of trophic factors or manipulating (e.g., gene therapy) the donor cells to secrete growth factors, such as substances directed towards stimulating angiogenic, vasculogenic, or neurogenic levels in the stroke brain. Proper regulation of these genetically engineered cells, as well as the optimal regimen for exogenous infusion of trophic/growth factors remain as major research endeavor in cell therapy validation studies.

Amnion Cells as Alternative Graft Source for Stroke. Amnion membrane-derived cells have three major features that make them appealing as donor source for cell therapy. First, the early origin of fetal membranes, which begin to develop even before gastrulation, gives rise to the possibility that cells in amniotic membrane maintain the plasticity of pregastrulation embryo cells. In particular, ES cells derive from the inner cell mass of the blastocyst, which gives rise to both the epiblast and the hypoblast. From this epiblast derive the three germ layers of the embryo and, from the epiblast, on about the eighth day after fertilization, derive the amniotic epithelial layer (amniotic ectoderm). The mesenchymal
Table 2. Status of Amnion and Other Cell Sources for CNS Transplantation

<table>
<thead>
<tr>
<th>Donor Cell</th>
<th>Current Application</th>
<th>Disease Indication</th>
</tr>
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<tbody>
<tr>
<td>Amnion</td>
<td>Preclinical studies</td>
<td>Stroke, Parkinson’s disease, spinal cord injury</td>
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<tr>
<td>Adipose tissue</td>
<td>Preclinical studies</td>
<td>Stroke, Parkinson’s disease, spinal cord injury</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Preclinical studies</td>
<td>Stroke, Parkinson’s disease, Huntington’s disease, Spinal cord injury</td>
</tr>
<tr>
<td>Embryonic tissue</td>
<td>Preclinical studies</td>
<td>Stroke, Parkinson’s disease, Huntington’s disease, Spinal cord injury</td>
</tr>
<tr>
<td>Fetal tissue</td>
<td>Clinical trials</td>
<td>Parkinson’s disease, Huntington’s disease</td>
</tr>
<tr>
<td>Gene transfected neural stem/progenitor cells</td>
<td>IND submission</td>
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<td></td>
<td>IND submission</td>
<td>Stroke</td>
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<tr>
<td>NT2N cells</td>
<td>Phase IIb</td>
<td>Stroke</td>
</tr>
<tr>
<td>Umbilical cord blood</td>
<td>Preclinical studies</td>
<td>Stroke, Parkinson’s disease, Huntington’s disease, Spinal cord injury, Amyotrophic lateral sclerosis, Multiple sclerosis</td>
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</tbody>
</table>

...stromal cells originate from extraembryonic mesoderm of the primitive streak (48). Secondly, the amnion has an anti-inflammatory function and immature immune system. Both hAEC and hAMC express various angiogenic and anti-inflammatory proteins such as interleukin (IL)-1 receptor antagonist; tissue inhibitors of metalloproteinase (TIMPs)-1, -2, -3, -4; and IL-10 (23).

In addition, amniotic membrane stromal matrix markedly suppressed lipopolysaccharide-induced upregulation of both IL-1α and -1β in human corneal limbal epithelial cells cultivated on it (69). Accordingly, the amnion has been considered to be suitable tissue or cell source for allograft transplantation, based on its anti-inflammatory effects, low immunogenicity as demonstrated by engraftment after xenotransplantation (2), and potential in modulating T-cell proliferation as demonstrated in vitro test of mixed lymphocytes culture (41). Thirdly, there was no evidence of tumorigenicity when isolated human amniotic cells were transplanted into animal models (Parolini, personal communication) or in human volunteers to examine their immunogenicity or into patients in an attempt to correct lysosomal storage diseases (1,57,66). However, the use of amnion cells for transplantation remains in its infancy and their efficacy and safety profile need similar preclinical validations as noted above for bone marrow-derived cells, before they are introduced in stroke clinical trials (Table 2).

**CONCLUSION**

The optimal cell transplantation regimen for stroke is under experimental investigation. Both embryonic stem cells and adult stem cells are being tested for their efficacy and safety in a variety of stroke animal models. The amnion, its membrane and fluid, is the most recent addition to the list of stem cell sources. Accumulating evidence of the amnion’s expansion and differentiation along pluripotent properties suggests that this cell population represents an efficacious transplantable source for cell therapy. Further studies are warranted to fully characterize the potential of amnion cells for treating stroke and other CNS disorders.

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