Published in final edited form as:

Future Neurol. 2015 ; 10(4): 321–326. doi:10.2217/FNL.15.19.

Stroke therapy: the potential of amniotic fluid-derived stem cells

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Keywords

placenta; regenerative medicine; stem cells; tissue engineering; transplantation

This article describes the clinical potential of cells harvested from human amniotic fluid in regenerative medicine, especially for stroke therapy. Amniotic fluid has been investigated as a new cell source for mesenchymal stem cells in the development of future cell-based transplantation. In this paper we highlight the characteristics of amniotic fluid-derived cells, as well as the functional benefits of these cells in animal models of stroke, altogether supporting the utility of amniotic fluid as stem cell source for cell therapy in stroke. The human amnion and amniotic fluid have attracted attention in recent years as possible reserves of stem cells that may have clinical application in the field of regenerative medicine. Several studies have examined the differentiation potential of cells derived from these two sources and concluded that they display high plasticity [1]. Currently, most studies have focused on cells derived from the amnion and revealed that amnion cell transplantation promotes re-epithelialization, modulates differentiation and angiogenesis and decreases inflammation, apoptosis and fibrosis [1–4]. This paper seeks to highlight what is currently known of the lesser studied, amniotic fluid-derived stem cells (AFSCs), and to acknowledge their potential clinical application for stroke therapy. Additionally, we will compare the advantages and disadvantages of amniotic fluid versus amnion membrane stem cells.

Stemness of cells derived from the amniotic fluid

The expression of specific pluripotency markers and genes in cells harvested from the amniotic fluid characterize these cells as stem cells. In a study by Antonucci *et al.* [5],

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None of these funders had role in the preparation and approval of contents of this manuscript. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

molecular analysis of human second trimester AFSCs was found to express *Fragilis*, *Stella*, *Vasa*, *c-Kit*, and *Rnf17*, genes involved in early stages of germ cell development, while also expressing *OCT4* and *SOX2*, markers of pluripotency. When AFSCs are aggregated to form embryoid bodies (EBs), they reacquire pluripotency potential and features of early stage embryogenesis that are usually lost [5]. Moreover, cells from AFSC-derived EBs express alternate spliced exons specific of pluripotent stem cells, such as the exon 10 of *DNMT3B* and the b isoform of *Sall4*; express markers of the three embryonic germ layers, such as *GATA4*, *GATA6*, *AFP* and *Nestin*; and there appears to be an absence of the X chromosome inactivation [5]. This observation further supports the important role of AFSCs in embryogenesis, as the reactivation of the inactive X chromosome may be correlated with genomic reprogramming events [5]. CD117-negative populations of human amniotic fluid mesenchymal stromal cells (AFMSCs) are readily abundant and can be easily reprogrammed into induced pluripotent stem cells (iPSCs) using nonintegrating Sendai viral vectors encoding for *OCT4*, *SOX2*, *KLF4* and *cMYC* [6]. Furthermore, Jiang *et al.* [6] have demonstrated that these iPSCs were virtually indistinguishable from human embryonic stem cells in multiple assays, could be used to generate a relatively homogeneous population of neural progenitors, and show engraftment potential *in vivo*. *In vitro*, these neural progenitor cells were shown to be capable of differentiating into mature neurons and astrocytes [6].

AFMSCs also possess gene expression profiles that are largely characteristic of undifferentiated cells [7]. In a similar study by Antonucci *et al.* [7], RT-PCR analysis showed that AFMSCs express genes for *Rex-1*, *SCF*, *GATA-4*, *vimentin*, *CK18*, *HLA ABC* and *FGF-5* throughout the culture period, and they express genes for *BMP-4*, *nestin*, *AFP* and *HNF-4 α* . As these genes regulate a multitude of different cell types, these observations suggest that AFMSCs are able to differentiate into adipocytes, osteocytes, chondrocytes and neuronal cells; can express many pluripotent stem cell specific genes; and proliferate well during *ex vivo* expansion [7].

While AFSCs have raised much interest because of their ability to differentiate *in vitro* into lineages belonging to all three germ layers, their immune properties are still being assessed. AFMSCs have been regarded as cells with low immunogenicity. Studies have observed AFMSCs to be resistant to rejection because they express immunosuppressive factors such as *CD59* (protectin) and *HLA-G* [8]. *CD59* inhibits the complement membrane attack complex by binding *C5b678* and hampering *C9* from binding and polymerizing, thus preventing complement from damaging cells [8]. *HLA-G*, which is expressed in the placenta unlike *HLA-A* and *HLA-B* genes, plays a key role in immune tolerance in pregnancy, [8]. Other recent studies have shown immunomodulatory properties of AFMSCs, which can inhibit the proliferation of T lymphocytes [8]. In another study, cultured AFSCs demonstrated an increase in *CD105*⁺ cells in the late-passage compared with the early-passage AFSC cultures [9]. Because *CD105* is a mesenchymal marker and the long-term culture conditions allowed mesenchymal cell growth, AFSCs have been suggested to be mesenchymal precursors [9]. Recent *in vitro* analysis has found that AFSCs modulate lymphocyte proliferation in different manners according to gestational age (i.e., those derived from first-, second- or third-trimester) [10]. Interestingly, first-trimester AFSCs significantly inhibited T cell and natural killer cell proliferation, while second- and third-

trimester AFSCs were less efficient, and only inflammatory-primed second-trimester AFSCs could suppress B-cell proliferation [10].

The preceding studies demonstrate that AFSCs exhibit characteristics of both embryonic and adult stem cells and vary from donor to donor [11]. Moreover, protein expression in the cell types found in AFMSCs does not affect the differentiation capacity of AFMSC preparations (PMID: 25608581), and the ectopic expression of Oct-4 in hAFMSCs could be an alternative method to produce pluripotency [12], while the selective expression of SOX9 and induction of Wnt signaling can be used to specifically differentiate cells to neurons and promote neurogenesis, respectively [13,14]. However, before these methodologies can be used, it is important to identify a suitable cryopreservation protocol, such as the slow-freezing solution [15]. In a recent study, Zong *et al.* has shown to direct AFSCs to differentiate into neurons with characteristics of functionality using inner stem cells derived as a feeder layer [16]. The study also showed that the Wnt signaling pathway plays an important role in triggering neurogenesis [16]. Thus, with their multifaceted properties, these cells have an important application in stroke therapy.

Transplantation studies using amniotic fluid stem cells for stroke therapy (Maya)

As of 2010, stroke is the fourth-leading cause of death among adults in the USA, accounting for about one of every 19 deaths [17]. Currently, the only nationally approved treatment for acute ischemic stroke is intravenous recombinant tissue plasminogen activator, a thrombolytic, within a narrow 3-h window of symptom onset. Thrombolytic therapy has been shown to significantly reduce the proportion of deaths and dependence in activities of daily living [18]. However, thrombolytic therapy also carries an increase in the risk of death within the first 7–10 days, intracranial hemorrhage and death at a 3- to 6-month follow-up [18]. Intravenous delivery of bone marrow- and perinatal-derived cells, which have the capacity to translocate to areas of tissue injury and target brain remodeling, may be a therapeutic intervention within the first week after stroke, during the restorative phase [19]. Stroke is a time-limited acute injury; the brain may be more favorable to transplantation, than in other organ or systemic diseases characterized by ongoing degenerative processes or immunological attacks [20].

Neurons are derived from the ectoderm lineage. Previous studies have demonstrated the ability of amniotic fluid-derived stem cells to differentiate along a neurogenic pathway [21,22]. Transplantation of cells derived from the amniotic fluid has been explored in neurological disorders [7,23–25]. Transplantation of AFSCs may serve as a promising option for stroke therapy; however, there are only a few studies that focus on these cells as a source for transplantation in stroke. The goals of AFSC transplantation post-stroke are to promote restorative mechanisms, such as neurogenesis, angiogenesis and immunomodulation, and to contribute to functional improvement [20].

One study had investigated the effect of AFSCs on focal cerebral ischemia-reperfusion injury and its consequential behavioral deficits in a mouse model. This study employed middle cerebral artery occlusion for 60 min, followed by a reperfusion phase for 7 days, to

produce this injury. Intracerebroventricular administration of amniotic fluid derived stem cells significantly reduced the neurological sequelae and behavioral deficits. The investigators also concluded that the beneficial effects of the AFSCs are comparable to those of embryonic neuronal stem cells, which typically carry ethical concerns [26].

Preclinical data of AFSC safety and efficacy suggests that individuals who suffer a stroke and show significant inflammation of the brain, or who display short-term memory loss due to the accompanying injury to the hippocampus, may be ideal candidates for future clinical trials of AFSC transplantation [27]. In addition, clinical trials of AFSC transplantation could be extended to patients with stroke of cardiovascular etiology, as preclinical data show substantial improvement in cardiac function following AFSC transplantation, suggesting that these cells are cardioprotective [28]. The administration route of AFSC transplantation may be dictated by the phase of stroke, that is, acute or chronic. Moreover, cryopreserved cells will also be required to ensure the ready availability of AFSCs for transplantation in both acute and chronic phases of stroke [27].

Our laboratory has tested the effect of amniotic fluid stem cell treatment in cerebral ischemia–reperfusion injury after stroke in rats [29]. Each rat was subjected to a series of behavioral tests to reveal neurological abilities prior to MCA occlusion, post-MCA occlusion and following transplantation of amniotic fluid-derived cells at day 35. Behavioral tests included the elevated body swing test and the rotarod test. We concluded that AFSC transplantation attenuates deficits in memory and learning, decreases infarct volume and neuron loss and increases cell proliferation [29].

Advantages & disadvantages of amniotic fluid versus amnion membrane stem cells

Both AFSCs and amnion membrane-derived stem cells (AMSCs) have their own advantages and disadvantages. Figure 1 summarizes the advantages and disadvantages of the two sources of stem cells. First, amniotic fluid can be collected during amniocentesis, while the amnion membrane can only be harvested after childbirth. This is a critical difference between the two sources. The early harvest of amniotic fluid allows the AFSCs to be isolated, cultured and amplified prior to childbirth. Thus, if the child develops any disease during or immediately after delivery, such as hypoxia, then the baby's own stem cells are readily available for use. On the other hand, it takes weeks after delivery to amplify AMSCs from amnion membrane. The critical therapeutic window might have passed when ample supply of stem cells from amnion membrane is available for transplantation. Second, autologous transplantation is more feasible with amniotic fluid than amniotic membrane due to the former's early harvesting period (i.e., during amniocentesis). Hence the child can benefit from his/her own stem cells. With amniotic membrane, allogeneic transplantation is more realistic than autologous, again due to the time required to generate sufficient amount of stem cells. Third, safety issues with amnion fluid and membrane need to be considered. With AFSCs requiring amniocentesis, there may be risks associated with such procedure that can cause injury to the mother and/or the child. In contrast, AMSCs can be easily collected after childbirth, posing no harm to the child and the mother. For this reason, harvesting stem cells from the amnion membrane is a much safer procedure compared with

amniotic fluid collection. Alternatively, the amniotic fluid can also be collected after childbirth, but the advantages mentioned above (e.g., readily available autologous AFSCs for transplantation during childbirth) are diminished. Fourth, the amnion membrane contains more stem cells compared with the amniotic fluid. Therefore it is easier to culture and amplify stem cells from amnion membrane than stem cells from amniotic fluid. However, because the amniotic fluid can be collected earlier using amniocentesis, there is plenty of time to amplify even with a smaller initial number of stem cells. Finally, it is difficult to isolate and confirm the stem cells' lineage of the amniotic fluid. AFSCs have to be phenotypically characterized to obtain a homogeneous cell population. Nonetheless, AFSCs have been shown to differentiate into multiple lineages [5,30–32]. On the other hand, the origin of amnion membrane is well studied. Most of the stem cells from amnion membrane are of epithelial and mesenchymal origins [33]. Hence, it is easier to isolate and direct further differentiation with AMSCs. However, recent studies have shown that most of the therapeutic effects of stem cells are from the secreted trophic factors, rather than the regenerated/differentiated stem cells [25,34]. Thus, a homogenous cell population may not be required as long as therapeutic outcomes are achieved.

Tissue engineering & regenerative potential of amniotic fluid stem cells

There is a possibility of harnessing the therapeutic properties of AFSCs and AFMSCs and applying them to tissue engineering and regenerative medicine for stroke. Studies from a relevant neurological disorder, traumatic brain injury (TBI), suggest the potential of AFSCs/AFMSCs as subdural patch-like network called biobridges. Biobridge formation may be fostered by AFMSC, as seen in notch-induced human bone marrow derived mesenchymal stromal cells during regeneration in a rat TBI model [35]. The formation of biobridges allows movement of exogenous and endogenous stem cells across non-neurogenic tissue to the site of injury [35]. This novel biobridge-based tissue engineering, coupled with the ability of AFMSC to differentiate into neural progenitor cells, may suppress the damaging inflammation. Moreover, the graft-host integration following the biobridge formation occurs without eliciting an immune response, advancing the notion of an amniotic fluid subdural patch as a promising therapeutic agent for regenerative medicine.

Xenografts have become a potential treatment for many health disorders, including neurological diseases. However, cells, tissues and organs transplanted from one species to another are, in many cases, rejected by the host's immune system. Xenoreactive antibodies lead to complement activation and systemic inflammation, rejecting the xenograft immediately [36,37]. Even immune tolerance techniques such as neonatal desensitization have failed to improve cell survival [38]. However, previous studies using circulating anti-inflammatory alpha-1-antitrypsin (ATT) in combination with anti-CD4/CD8 therapy have shown to protect xenografts from inflammation, and as a result, promote xenograft acceptance [39]. With this in mind, AFMSC could be used as a new strategy to augment xenograft rejection. Co-transplantation of immunosuppressive amniotic fluid stem cells may then augment xenograft rejection.

In addition to dampening the immune and inflammatory response associated with xenografts, AFMSCs may also enhance the therapeutic outcome of allograft transplantation.

Allograft tolerance can be described as the absence of a destructive reaction by the host's immune system against alloantigens of the transplant. The early phase of the immune response in allograft rejection is the innate and non-specific immune response. The subsequent phase is the donor-specific adaptive immune response, which results from T-lymphocyte recognition of the alloantigens [40]. The immunomodulatory properties of AFMSCs may help to promote long-term allograft acceptance and reduce chronic immunosuppression [8,41].

Conclusion

Although more is known about cells derived from the amniotic membrane than from amniotic fluid, there is great potential for future clinical application of AFSCs. The routine access to the amniotic fluid either during amniocentesis or after birth, easy isolation and amplification of stem cells, ability to differentiate into many cell lines, capacity to exert immunomodulatory effects and lack of ethical barriers associated with AFSCs make them an ideal cell source. AFSC transplantation could be utilized in the treatment of ischemic stroke, by promoting neurogenesis, angiogenesis and immunomodulation. Further research needs to be done to determine the full therapeutic spectrum of AFSC, along with identifying the optimal timing and best administration route for transplantation in clinically relevant stroke models. Exploring the potential of these cells could lead to great advances in the fields of tissue engineering and regenerative medicine, and eventually toward the clinical application of these cells in stroke.

Acknowledgments

Financial & competing interests disclosure: CV Borlongan is supported by National Institutes of Health, National Institute of Neurological Disorders and Stroke 1R01NS071956-01, Department of Defense W81XWH-11-1-0634, James and Esther King Foundation for Biomedical Research Program, SanBioInc, KM Pharmaceuticals, NeuralStemInc and Karyopharm, Inc.

Biography

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Amniotic fluid-derived stem cells	Amnion membrane-derived stem cells
Advantages <ul style="list-style-type: none">• Can be harvested through amniocentesis• Cells are ready at the time of childbirth• Autologous transplantation is more feasible	Advantages <ul style="list-style-type: none">• Safer harvesting procedure• More stem cells can be harvested• Well-studied origins of the stem cells = easier to direct further differentiation
Disadvantages <ul style="list-style-type: none">• Associated risks with amniocentesis• Less stem cells compared to the amnion membrane• Difficult to isolate and direct further differentiation	Disadvantages <ul style="list-style-type: none">• Can only be harvested after delivery• Takes weeks after childbirth to amplify cells• Allogenic transplantation more realistic

Figure 1.

Advantages and disadvantages of amniotic fluid-derived stem cells and amnion membrane-derived stem cells.