

Amniotic Fluid-Derived Stem Cells (AFSC) and Their Application in Cell Therapy and Tissue Engineering

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Context: Amniotic Fluid Derived Stem Cells (AFSC) has mesenchymal origin and is multipotent. Having played their role in the detection of genetic abnormalities in the unborn children, they are gaining attention in the regenerative medicine because of their pluripotency.

Evidence Acquisition: AFSCs possess great proliferating ability and have no ethical and religious issues in their use. AFSCs may also be studied for the stem cells differentiation such as production of multiple lineages of different cells like heart, liver, pancreas, etc. The potential of their use in regenerative medicine as well as their differentiation into multiple cells is possible.

Results: AFSCs have the potential to be used in tissue repair and regeneration of bladder and kidney injuries, for the treatment of congenital anomalies like tracheal anomalies and spina bifida therapy etc. However, like every therapeutic potential, AFSCs also have some limitations such as low rate of differentiation of transplanted AFSCs and immune rejection.

Conclusions: AFSCs have great therapeutic potential, but extensive research is warranted to overcome the limitations to use AFSC as therapy.

Keywords: Regenerative Medicine; Amniotic Fluid; Stem Cells; Graft Rejection

1. Context

Amniotic Fluid Stem Cells (AFSCs) may be isolated from amniotic fluid and are mesenchymal in origin. They are multipotent and non-tumorigenic when injected in vivo (1, 2). Human amniotic fluid has been used in clinical application such as prenatal diagnosis for more than 70 years and the diagnostic procedure is safe, simple and reliable. Many types of fetal developmental and genetic disorders such as chromosome abnormalities, down syndrome (trisomy 21), trisomy 13, trisomy 18, fragile X, neural tube defects and rare inherited metabolic disorders (anencephaly and spina bifida) may be diagnosed by screening of amniotic fluid as well as all the components and cells presented in it (3). Recent studies show that amniotic fluid is much more than a simple diagnostic tool. AFSCs may be derived from amniotic fluid without the destruction of embryo by amniocentesis (4). Human amniotic fluid stem cells also express the OCT 4 transcription factor that is the stemness marker for embryonic stem cells (5) and is also an important factor for the induced pluripotency (6). These cells are selected by the CD 117 (surface antigen c-kit) cell

surface marker expression and the type III tyrosine kinase receptor (7, 8).

hAFSCs may have the greater differentiation potential as they express OCT 4 as well other pluripotent markers such as SSEA- 4, CD 29, CD 44, CD 73, CD 90, CD 105, and CD 133 (9). However, further research is warranted to explore their pluripotency. These cells can proliferate highly when cultured in vitro with a doubling time of 1.6 days (10) and do not require feeder cells during growth in culture (1, 5). In addition, there are no ethical, political and religious issues in the use of these cells as they do not disrupt the embryo as compared to the derivation of human embryonic stem cells (ESCs) (4). AFSC may be an alternative to ESCs as they do not form teratoma (11) and have the capability to differentiate into broad spectrum of lineages (12).

As AFSCs have the characteristics of self-renewal and differentiation into diverse mature progeny, they are considered as suitable mean for use in cell based therapies and tissue repair. AFSCs are being used for the development of therapies for many preclinical models of disease and injury (13).

2. Evidence Acquisition

2.1. Origin of AFSC

AFSCs are of mesenchymal origin (11) and derived from the amniotic fluid that is present in the amnion. Amnion is the innermost extra-embryonic membrane that surrounds the fetus. AFSCs have been used for genetic diagnoses for long ago but its origin has been recently investigated (14). These cells have both embryonic and extra embryonic origins. Rosner (2013) recently declared that precise origin of AFSCs is still unknown. They showed that these pluripotent stem cells float in the amniotic fluid during pregnancy, and the related *in vivo* significance is still unknown. It has been assumed that these AFSCs and the floating fetal cells may have some common origins (15).

2.2. AFSC and Health Status Defects in an Unborn Child

During amniocentesis, the amniotic fluid drawn out is used in prenatal diagnosis of the fetus genetic abnormalities and infections (diagnostic tests). Many malformations such as cardiovascular disorders may be detected by ultrasound while genetic diagnosis is done through amniotic fluid derived cells. Generally amniocentesis is performed, if it is suspected that the unborn child has some genetic defects such as Down syndrome etc. (8).

3. Results

3.1. Endogenous Tissue Repair

Because of their differentiation potential, AFSCs have great potential in future tissue engineering and cell based therapies. These cells have some unique and important features that can lead to the successful tissue regeneration and repair (16, 17). Studies show that in the sciatic nerve crush, AFSCs have improved electrophysiological indicators of nerve and motor functions (18). It was shown in the studies by Pan et al. that the enhanced nerve regeneration was due to the secretion of neurotrophic factors from amniotic fluid mesenchymal stem cells (MSCs) (19). In mice, AFSCs injection also improved the memory, sensory and motor functions after local ischemia induced by Middle Cerebral Artery Occlusion (MCAO) (20).

It was first demonstrated that AFSCs could be used for tissue engineering by Kaviani, et al., 2001. Since then, AFSCs have been used in experiments for the tissue repair including heart valve leaflets, repair of tendons for diaphragm, grafts of bone and cartilage grafts for fetal tracheal reconstruction (21, 22). When AFSCs administered *in vivo*, they showed improvement in many injury models including bladder, hyperoxic lung and kidney injuries etc. (11, 23, 24).

3.2. Treatment of Congenital Anomalies

As mentioned above, amniotic fluid-derived stem cells have the potential to treat many types of congenital abnormalities. Congenital abnormalities can be cured or corrected by creating the tissues from the baby's own cells. For this purpose, fetal stem cells or AFSC are used which have the potency to differentiate into many types of cells. Figures 1 and 2 summarize the use of these stem cells for the treatment of congenital defects as well as for injuries and degenerative diseases. Stem cells therapy can be used to repair damaged tissues that are difficult to treat by the conventional therapies (25).

3.3. Cell Fate Specification and Regenerative Medicine

AFSCs can be used for the investigation of the factors that control cell fate as some studies have been carried out on the investigation of cellular behavior and determination of signaling pathways that control the cell fate. AFSCs are promising cellular models in studying the role of signaling pathways in the homeostasis of stem cells (8). Furthermore, AFSCs have the capability to differentiate into multiple lineages under specific culture conditions such as heart, lung, hematopoietic, pancreas, liver, bone, chondrocytes, adipose tissue and skeletal muscular. In addition, AFSCs also act as cytokines regulators (26).

Differentiation of AFSCs in neural cells was tested by investigating the CNS development in mutant new born mice. Mutant mice lacked the lysosomal enzyme galactocerebrosidase and undergo neurological deterioration. After the induction of AFSCs along with the nerve growth factors, these stem cells were injected in to the lateral cerebral ventricles of the brains of mice. It was seen after a month, the number of engrafted human AFSCs in mutant mice were higher as compared to that of wild type. AFSCs control the process of neurogenesis that lead to the improvement of the disorder (1).

De Coppi et al. (2007) also worked on the osteogenic lineage of AFSCs. These cells were cultured in a medium that differentiated the cells in osteogenic lineage. The cells were able to produce mineralized calcium and secreted alkaline phosphates (ALP). In addition, when these cells were injected into immunodeficient mice, they produced mineralized tissues in mice (1). Different research groups are also working on AFSCs for the regeneration of kidney. They have shown that AFSCs can form renal structures and help in renal tissues regeneration. These cells express specific kidney cell markers and differentiate into glomerular and tubular structures (27).

Another research group obtained heart valves leaflets from AFSCs (26). Another research group injected the human AFSCs in ischemic myocardium of normal mice. In this case, immune rejection of xeno-transplanted cells was observed (28), however; it has been demonstrated that AFSCs have the potential to differentiate into functional cardiomyocytes both *in vivo* and *in vitro* (29).

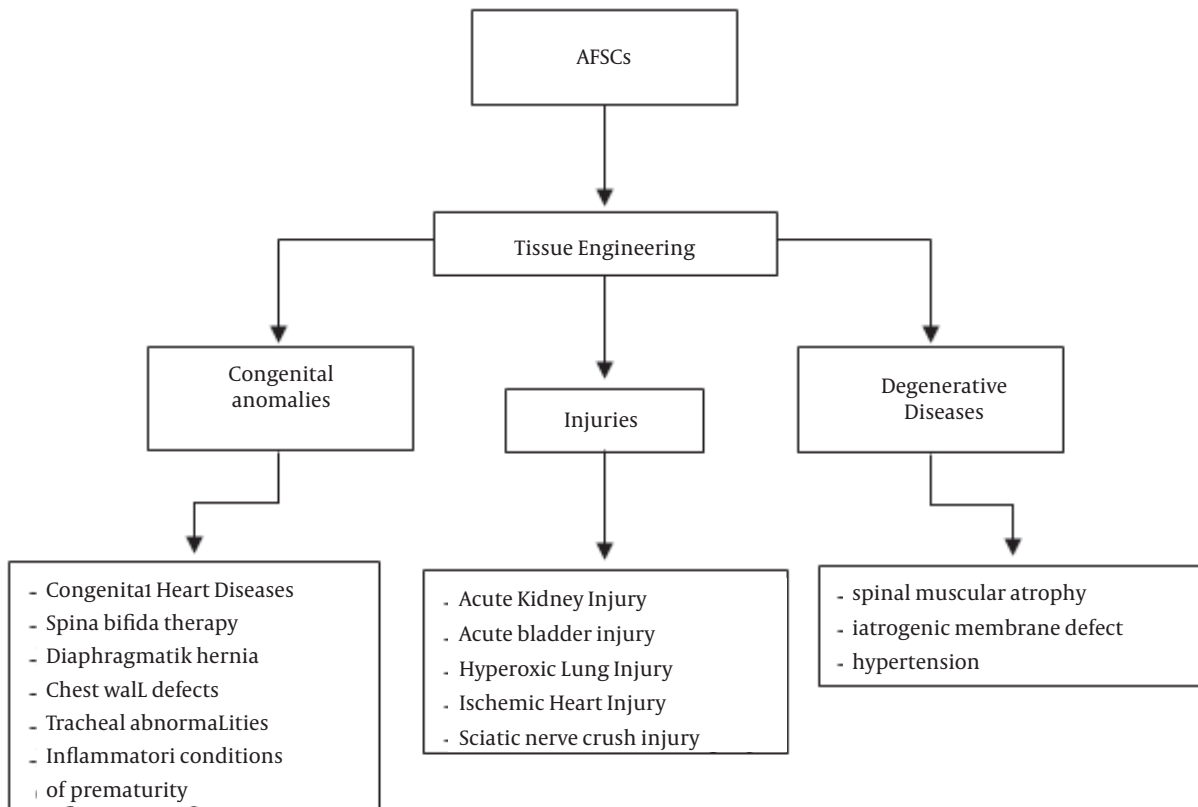
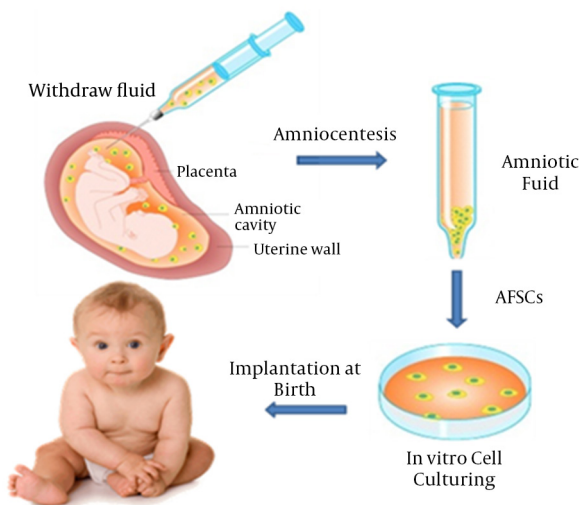


Figure 1. Applications of AFSCs for the Treatment of Various Diseases

Figure 2. Treatment of Congenital Abnormalities



AFSCs Can be obtained by amniocentesis and are cultured in vitro in a way that they grow in parallel with the remaining time of gestation. Finally, these cells are placed on biodegradable scaffolds and are implanted in child after birth (Shaun et al. 2012).

Bollini et al. (2011) proved the role of AFSCs in in vivo cardio-protection after the myocardial infarction (MI) (29). Bollini and his fellows administered the hAFSCs to the mice with ischemia and infarction. They analyzed the infarct size and secretion of paracrine factor thymosin β 4 ($T\beta$ 4) by assessing 2, 3, 5-triphenyltetrazolium chloride staining assay and enzyme-linked immunosorbent assay respectively. Induction of hAFSCs showed decreased infarct size and increasing $T\beta$ 4 secretion that shown to be both proangiogenic and cardio-protective (29). The differentiation potential of AFSC may lead to the therapy for degenerative heart disorders as well as congenital heart diseases (30).

AFSCs also have the potential to differentiate into hepatic lineage. But only few studies have been reported in this regards. These cells can be used in regeneration of liver (31). More work is needed to investigate the use of AFSCs for hepatocyte differentiation.

3.4. AFSCs Banks

AFSCs have high proliferation rate and these cells have the capability to be stored for longer periods of time (32). The banking of AFSCs will guarantee a source of stem cells that will have the matching immune profile with

the recipient as they can be used anytime later in life (1). As AFSCs can renew themselves and their banking is possible, so they can be considered as well characterized, stable cells for many types of cell therapies and tissue engineering (33).

AFSCs are very much suitable for large scale banking because they have high capacity for expansion and have comparable immunomodulatory capability (1). These cells can be stored in a tissue bank or cell culture bank, like cord blood banks and can be used in future for congenital anomalies and in regenerative medicine (34). Many private companies offer the services to preserve the amniotic fluid derived stem cells for a fee like Biocell Center in Medford, Massachusetts, USA.

Studies reveal that a AFSCs bank with 100,000 specimens theoretically can supply 99% of the United States (U.S.) population with perfect genetic matches for transplantation. In U.S, there is more than four million per annum live births which can provide an extensive resource for extraction of AFSCs (4).

3.5. Advantages of AFSCs in Regenerative Medicine

3.5.1. Isolation

It is easy to isolate and harvest AFSCs from amniotic fluid as these cells are readily accessible. A very rich population of AFSCs can be obtained from amniotic fluid. AFSCs can also be isolated from the gestational tissues such as placenta, amniotic fluid and placental membranes that are usually discarded after birth (35). In addition, they can be derived from amniotic fluid which is isolated during amniocentesis, a process used during pregnancy for the evaluation of the health status of the fetus (3). Apart from amniotic fluid, AFSCs can be isolated from placenta and umbilical cord at birth (36-39).

3.5.2. High Proliferative Rate

In contrast to MSCs that have limited growth potential, AFSCs have some advantages over MSCs (33). These cells have unique characteristics that are major focus of recent researches. As compared to adult stem cells, AFSCs have high proliferative activity and the doubling time of 1.6 days or 36 hours and they may be grown in absence of feeder cells (1, 40).

3.5.3. Higher Differentiation Potential

AFSCs have higher differentiation potential. Their potential to differentiate into variety of lineages has made it an attractive tool for their use in therapeutics and regenerative medicine (40).

3.5.4. Genetically Stable

AFSCs are genetically stable and do not cause somatic or epigenetic mutations which iPS cell can cause. Due to

the induction of pluripotency in iPS, these cells harbor the somatic mutations and do not exhibit the perfect epigenetic memory of the source cell (41) while, AFSCs do not require pluripotency induction. In contrast to MSCs from bone marrow which undergo faster differentiation, AFSCs can maintain and increase the mineralization of bone for longer time (42). Furthermore, the pattern of epigenetics persists in these cells (43). Due to these and other similar advantages, AFSCs can be used as a very important factor for the future research and treatment.

3.6. Limitations/Disadvantages

Advanced studies show that different methods for extraction of AFSCs and culture conditions lead to diverse subpopulations of cells. These subpopulations have different morphologies, growth kinetics and cell marker expressions (44, 45). It is still not clear that what factors affect the methodological differences. Furthermore, the phenotypes obtained after differentiation are affected by the gestational stage at which amniotic fluid collected (32) and by the passage number of the cultured cells (46). According to studies, AFSCs are not homogenous as they were supposed to be before (35).

3.6.1. Low Rate of Differentiation of Transplanted AFSCs

In order to do transplantation of cells, cells are usually differentiated into a certain phenotypes, but according to different studies, despite having high proliferation rate, AFSCs have very low differentiation rate which restrict their application in cell therapy (47).

3.6.2. Immune Rejection

Previously it was thought that AFSCs have low immune rejection. But recent studies show that they have low rate of survival after transplantation and the reason might be immune rejection (48). When AFSCs transplanted in immune-competent mice, the cells were rejected (28). It is essential to do more works on studying the immunological properties of AFSCs to increase the survival rate of these cells after transplantation.

4. Conclusions

According to the recent studies, AFSCs have high proliferation and multi-lineage differentiation potential making them an ideal cellular source for the treatment of congenital defects and other degenerative diseases. AFSCs display a phenotype that is in the middle of embryonic and adult stem cells making them superior than adult stem cells. Although AFSCs have a lot of advantages but there are still many limitations due to their slow differentiation and immune rejection. There is need for further studies on isolation, culture, and differentiation of AFSCs so that they can be used for tissue engineering and regenerative medicine.

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Authors' Contributions

Syeda Anum Zahra prepared the manuscript with the help of other authors under the supervision of Aftab Ahmad.

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