Razavi Int J Med. 2015 February; 3(1): e20135. DOI: 10.30483/rijm.2015.118375
Published online 2015 February 20. **Review Article**

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

Syeda Zahra Anum [1]; Seyed Raheel Muzavir [2]; Ahmad Hassan [3]; Amir Ali Khan [1,4]; Aftab Ahmad [2,*]

[1]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[2]National Academy of Young Scientists, School of Biological Sciences, University of the Punjab, Lahore, Pakistan
[3]Department of Biology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Neurosciences, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia

*Corresponding author: Aftab Ahmad, National Academy of Young Scientists, School of Biological Sciences, University of the Punjab, Lahore, Pakistan. Tel: +92-3007402202, Fax: +92-4299230980, E-mail: aftabac@yahoo.com

Received: May 10, 2014; **Revised:** November 1, 2014; **Accepted:** December 1, 2014

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-tumorogenic 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).

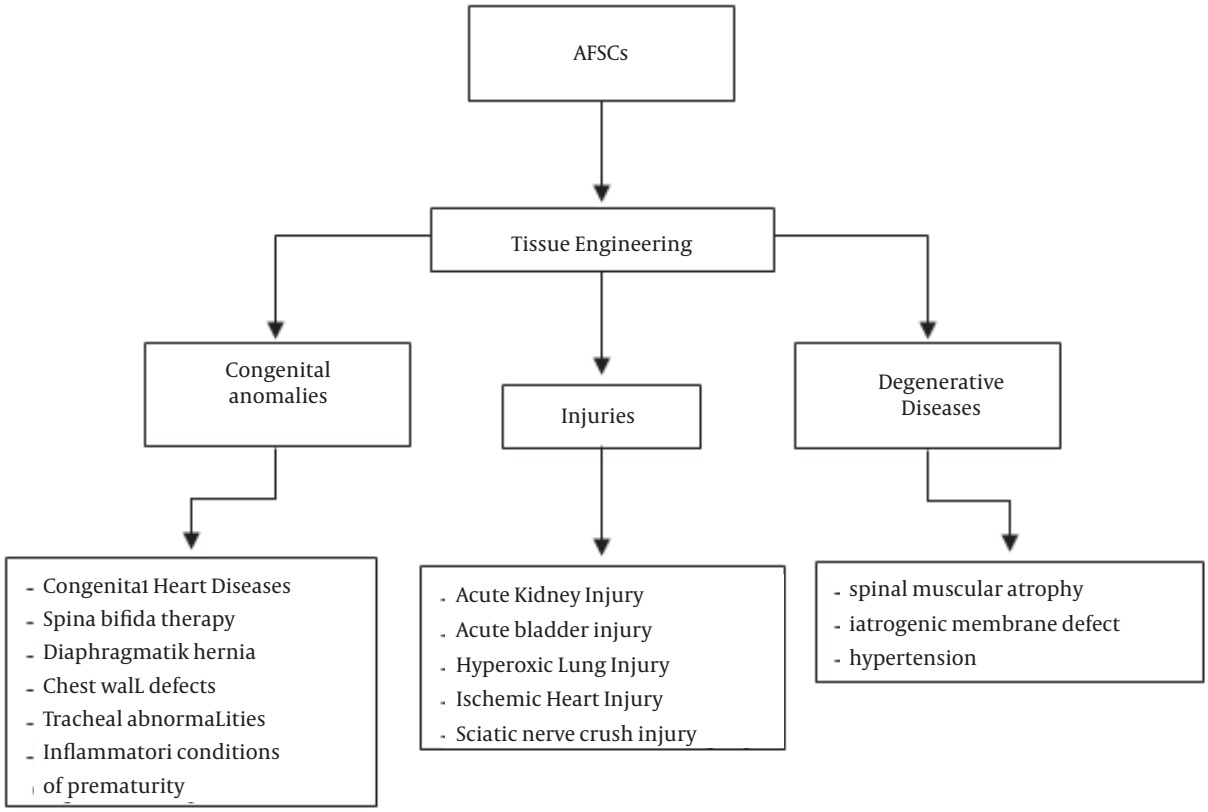
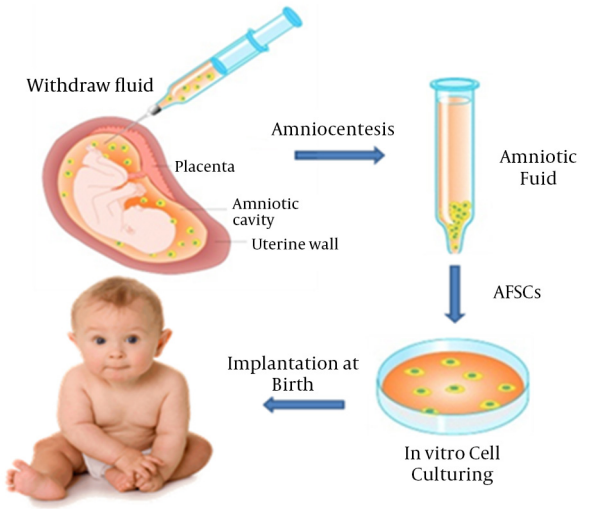


Figure 1. Applications of AFSCs for the Treatment of Various Diseases

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 (Tβ 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 Tβ 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 linage. 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 intro 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.

Acknowledgements

We are thankful to National Academy of Young Scientists (NAYS) Pakistan for providing us a network to connect with relevant scientists.

Authors' Contributions

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

References

1. De Coppi P, Bartsch GJ, Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol.* 2007;**25**(1):100–6.
2. Petsche Connell J, Camci-Unal G, Khademhosseini A, Jacot JG. Amniotic fluid-derived stem cells for cardiovascular tissue engineering applications. *Tissue Eng Part B Rev.* 2013;**19**(4):368–79.
3. Jeffrey SD, Sherman E. *Prenatal diagnostic testing.*: Merck Manual; 2008.
4. Atala A. Essentials of stem cells. In: Lanza JR, Gearhart B, Hogan D, Melton R, Pedersen ED, Thomas J, et al editors. *Amniotic Fluid-derived Pluripotent Cells.* 2nd ed. Canada: Elsevier; 2009.
5. Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschlager M. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? *Hum Reprod.* 2003;**18**(7):1489–93.
6. Guillot P, Moschidou D, Pascale VG. *Reprogramming human amniotic fluid stem cells to functional pluripotency by manipulation of culture conditions.*: Protocol Exchange; 2012. Available from: http://www.nature.com/protocolexchange/protocols/2426.
7. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, et al. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell.* 1990;**63**(1):213–24.
8. Klemmt P. Application of amniotic fluid stem cells in basic science and tissue regeneration. *Organogenesis.* 2012;**8**(3):76.
9. Phermthai T, Odglun Y, Julavijitphong S, Titapant V, Chuenwattana P, Vantanasiri C, et al. A novel method to derive amniotic fluid stem cells for therapeutic purposes. *BMC Cell Biol.* 2010;**11**:79.
10. Tsai MS, Lee JL, Chang YJ, Hwang SM. Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. *Hum Reprod.* 2004;**19**(6):1450–6.
11. De Coppi P, Callegari A, Chiavegato A, Gasparotto L, Piccoli M, Taiani J, et al. Amniotic fluid and bone marrow derived mesenchymal stem cells can be converted to smooth muscle cells in the cryo-injured rat bladder and prevent compensatory hypertrophy of surviving smooth muscle cells. *J Urol.* 2007;**177**(1):369–76.
12. Tsai MS, Hwang SM, Tsai YL, Cheng FC, Lee JL, Chang YJ. Clonal amniotic fluid-derived stem cells express characteristics of both mesenchymal and neural stem cells. *Biol Reprod.* 2006;**74**(3):545–51.
13. Murphy SV, Atala A. Amniotic fluid and placental membranes: unexpected sources of highly multipotent cells. *Semin Reprod Med.* 2013;**31**(1):62–8.
14. Dobreva MP, Pereira PN, Deprest J, Zwijsen A. On the origin of amniotic stem cells: of mice and men. *Int J Dev Biol.* 2010;**54**(5):761–77.
15. Rosner M, Hengstschlager M. Amniotic fluid stem cells and fetal cell microchimerism. *Trends Mol Med.* 2013;**19**(5):271–2.
16. Rodrigues MT, Lee SJ, Gomes ME, Reis RL, Atala A, Yoo JJ. Amniotic fluid-derived stem cells as a cell source for bone tissue engineering. *Tissue Eng Part A.* 2012;**18**(23-24):2518–27.
17. Tajiri N, Acosta S, Glover LE, Bickford PC, Jacotte Simancas A, Yasuhara T, et al. Intravenous grafts of amniotic fluid-derived stem cells induce endogenous cell proliferation and attenuate behavioral deficits in ischemic stroke rats. *PLoS One.* 2012;**7**(8).
18. Pan HC, Cheng FC, Chen CJ, Lai SZ, Lee CW, Yang DY, et al. Post-injury regeneration in rat sciatic nerve facilitated by neurotrophic factors secreted by amniotic fluid mesenchymal stem cells. *J Clin Neurosci.* 2007;**14**(11):1089–98.
19. Pan HC, Yang DY, Chiu YT, Lai SZ, Wang YC, Chang MH, et al. Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell. *J Clin Neurosci.* 2006;**13**(5):570–5.
20. Rehni AK, Singh N, Jaggi AS, Singh M. Amniotic fluid derived stem cells ameliorate focal cerebral ischaemia-reperfusion injury induced behavioural deficits in mice. *Behav Brain Res.* 2007;**183**(1):95–100.
21. Kaviani A, Perry TE, Dzakovic A, Jennings RW, Ziegler MM, Fauza DO. The amniotic fluid as a source of cells for fetal tissue engineering. *J Pediatr Surg.* 2001;**36**(11):1662–5.
22. Weber B, Emmert MY, Behr L, Schoenauer R, Brokopp C, Drogemuller C, et al. Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation. *Biomaterials.* 2012;**33**(16):4031–43.
23. Perin L, Sedrakyan S, Giuliani S, Da Sacco S, Carraro G, Shiri L, et al. Protective effect of human amniotic fluid stem cells in an immunodeficient mouse model of acute tubular necrosis. *PLoS One.* 2010;**5**(2).
24. Carraro G, Perin L, Sedrakyan S, Giuliani S, Tiozzo C, Lee J, et al. Human amniotic fluid stem cells can integrate and differentiate into epithelial lung lineages. *Stem Cells.* 2008;**26**(11):2902–11.
25. Anum SZ, Muzavir SR, Zafa H, Khan AA, Ahmad A. Stem Cells Therapy as Treatment for Spinal Cord Injury. *Health.* 2012;**3**(1):19–23.
26. Sacco SD, Roger E, De Filippo RE, Perin L. Tissue Engineering and Regenerative Medicine. In: Sabine WG editor. : Advances in Regenerative Medicine; 2011.
27. Perin L, Giuliani S, Jin D, Sedrakyan S, Carraro G, Habibian R, et al. Renal differentiation of amniotic fluid stem cells. *Cell Prolif.* 2007;**40**(6):936–48.
28. Chiavegato A, Bollini S, Pozzobon M, Callegari A, Gasparotto L, Taiani J, et al. Human amniotic fluid-derived stem cells are rejected after transplantation in the myocardium of normal, ischemic, immuno-suppressed or immuno-deficient rat. *J Mol Cell Cardiol.* 2007;**42**(4):746–59.
29. Bollini S, Cheung KK, Riegler J, Dong X, Smart N, Ghionzoli M, et al. Amniotic fluid stem cells are cardioprotective following acute myocardial infarction. *Stem Cells Dev.* 2011;**20**(11):1985–94.
30. Hilfiker A, Kasper C, Hass R, Haverich A. Mesenchymal stem cells and progenitor cells in connective tissue engineering and regenerative medicine: is there a future for transplantation? *Langenbecks Arch Surg.* 2011;**396**(4):489–97.
31. Saulnier N, Lattanzi W, Puglisi MA, Pani G, Barba M, Piscaglia AC, et al. Mesenchymal stromal cells multipotency and plasticity: induction toward the hepatic lineage. *Eur Rev Med Pharmacol Sci.* 2009;**13 Suppl 1**:71–8.
32. Da Sacco S, Sedrakyan S, Boldrin F, Giuliani S, Parnigotto P, Habibian R, et al. Human amniotic fluid as a potential new source of organ specific precursor cells for future regenerative medicine applications. *J Urol.* 2010;**183**(3):1193–200.
33. Moorefield EC, McKee EE, Solchaga L, Orlando G, Yoo JJ, Walker S, et al. Cloned, CD117 selected human amniotic fluid stem cells are capable of modulating the immune response. *PLoS One.* 2011;**6**(10).
34. Zhang P, Baxter J, Vinod K, Tulenko TN, Di Muzio PJ. Endothelial differentiation of amniotic fluid-derived stem cells: synergism of biochemical and shear force stimuli. *Stem Cells Dev.* 2009;**18**(9):1299–308.
35. Rennie K, Gruslin A, Hengstschlager M, Pei D, Cai J, Nikaido T, et al. Applications of amniotic membrane and fluid in stem cell biology and regenerative medicine. *Stem Cells Int.* 2012;**2012**:721538.
36. Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, Lombardi G, et al. Engraftment potential of human amnion and chorion cells derived from term placenta. *Transplantation.* 2004;**78**(10):1439–48.
37. Banas RA, Trumpower C, Bentlejewski C, Marshall V, Sing G, Zeevi A. Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. *Hum Immunol.* 2008;**69**(6):321–8.
38. Brunstein CG, Wagner JE. Cord blood transplantation for adults. *Vox Sang.* 2006;**91**(3):195–205.
39. Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. *Stem Cells.* 2004;**22**(5):649–58.

40. Rosner M, Schipany K, Shanmugasundaram B, Lubec G, Hengstschlager M. Amniotic fluid stem cells: future perspectives. *Stem Cells Int.* 2012;**2012**:741810.
41. Rosner M, Dolznig H, Schipany K, Mikula M, Brandau O, Hengstschlager M. Human amniotic fluid stem cells as a model for functional studies of genes involved in human genetic diseases or oncogenesis. *Oncotarget.* 2011;**2**(9):705–12.
42. Peister A, Woodruff MA, Prince JJ, Gray DP, Hutmacher DW, Guldberg RE. Cell sourcing for bone tissue engineering: amniotic fluid stem cells have a delayed, robust differentiation compared to mesenchymal stem cells. *Stem Cell Res.* 2011;**7**(1):17–27.
43. Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R, et al. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. *Cell Stem Cell.* 2011;**8**(1):106–18.
44. Zhang S, Geng H, Xie H, Wu Q , Ma X, Zhou J, et al. The heterogeneity of cell subtypes from a primary culture of human amniotic fluid. *Cell Mol Biol Lett.* 2010;**15**(3):424–39.
45. Roubelakis MG, Bitsika V, Zagoura D, Trohatou O, Pappa KI, Makridakis M, et al. In vitro and in vivo properties of distinct populations of amniotic fluid mesenchymal progenitor cells. *J Cell Mol Med.* 2011;**15**(9):1896–913.
46. Kim YW, Kim HJ, Bae SM, Kim YJ, Shin JC, Chun HJ, et al. Time-course transcriptional profiling of human amniotic fluid-derived stem cells using microarray. *Cancer Res Treat.* 2010;**42**(2):82–94.
47. Aurich H, Sgodda M, Kaltwasser P, Vetter M, Weise A, Liehr T, et al. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo. *Gut.* 2009;**58**(4):570–81.
48. Soler R, Fullhase C, Hanson A, Campeau L, Santos C, Andersson KE. Stem cell therapy ameliorates bladder dysfunction in an animal model of Parkinson disease. *J Urol.* 2012;**187**(4):1491–7.