THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (January–February 2018) 68(1):15–19
https://doi.org/10.1007/s13224-017-1034-z

INVITED MINI REVIEW

Amniotic Fluid Stem Cells: A New Era in Regenerative Medicine

Mala Srivastava[1] · **Neha Ahlawat**[1] · **Ankita Srivastava**[2]

Received: 24 May 2017 / Accepted: 3 July 2017 / Published online: 27 July 2017

About the Author

Dr. (Prof.) Mala Srivastava, Senior Consultant and Robotic Surgeon, Institute of Obstetrics and Gynaecology, Sir Ganga Ram Hospital is President ALUMNI Association (GRIPMER), and Secretary ISOPARB (Indian Society of Perinatology and Reproductive Biology) Delhi Chapter. She is chairperson of cervical cancer Awareness and Prevention, Subcommittee of AOGD and chairperson of Community Health Committee of ISOPARB. She is also executive member of ISCCP (Indian Society of Colposcopy and Cervical Pathology) and ISOPARB. She had been past joint secretary of ALUMNI Association (GRIPMER), AOGD and NARCHI twice. She has presented many papers in National and International Conferences with BEST PAPER AWARDS in ISOPARB 2003, AOGD 2005, AICOG 2007, and IMS 2008 (International Conference). She has published many papers in National and International Journals and contributed in chapters of Books. She had been peer reviewer for journals. She has organized many conferences as organizing Secretary. Her special interest is in gynae oncology and robotic surgery.

Abstract Regenerative medicine has become an emerging field which focuses on repair, replacement or regeneration of cells, tissues and the entire organs. The regeneration may occur in patient's own body by using their system as a bioreactor, e.g., cell therapy that involves transplantation of stem cells capable of proliferating, differentiating and replacing damaged host cells. As the field of regenerative medicine advances, and sources of stem cells has been intensified. Though embryonic and adult tissues can be used for isolation of pluripotent stem cells, the amniotic fluid (AF) has been proposed as an alternative source of stem cells for tissue regeneration. AF cells could be banked and used for either allogeneic or autologous transplantation.

Keywords Stem cell · Banking · Regenerative medicine · Amniotic fluid · Transplantation · Allogenic · Autologous

Dr. Mala Srivastava, Senior Consultant, Robotic Surgeon in Institute of Obstetrics and Gynaecology at Sir Ganga Ram Hospital, New Delhi; Neha Ahlawat, Research Associate, New Delhi; Dr. Ankita Srivastava, Senior Resident in Department of Obstetrics and Gynaecology at Lok Nayak Jay Prakash Hospital, New Delhi.

✉ Mala Srivastava
malasrivastava2001@yahoo.co.in

[1] Institute of Obstetrics and Gynaecology, Sir Ganga Ram Hospital, New Delhi, India

[2] Department of Obstetrics and Gynaecology, Lok Nayak Jay Prakash Hospital, New Delhi, India

Introduction

Regenerative medicine has become an emerging field which focus on repair, replacement or regeneration of cells, tissues and the entire organs. It involves multidisciplinary approach in relation to the regeneration process and includes stem cell biology, gene therapy, bioengineering, material science and pharmacology.

Scientists all over the world have been successful in tissue engineering preparing simple organs with functions of allowing passage (e.g. trachea) or storage (e.g. urinary bladder) in the body. But there are possibilities that regeneration may occur in patient's own body by using their system as a bioreactor, e.g. cell therapy which involves transplantation of stem cells capable of proliferating, differentiating and replacing damaged host cells [1], or activating or enhancing innate regenerative processes (e.g. transplantation of stem cells that work at sites of injury and act through a paracrine mechanism to stimulate repair/regeneration of host tissues) [2, 3]. Hence, regenerative medicine may offer a long-term solution to the problems of shortage of organs available for transplantation.

As the field of regenerative medicine advances, the search for sources of stem cells has been intensified with potential for therapy. Though embryonic and adult tissues can be used for isolation of pluripotent stem cells, the amniotic fluid (AF) has been proposed as an alternative source of stem cells for tissue regeneration. AF cells could be banked and used for either allogeneic or autologous transplantation.

In humans, the amnion is a sac containing the developing embryo, surrounded by the chorion and yolk sac. Along with the enveloping AF, it also protects the foetus, against trauma, infections and toxic agents [4]. The composition of AF and volume fluctuates with gestation, in association with the foetal development. During the early part of pregnancy, AF is dependent on the osmotic gradient formed by sodium and chloride transport across the amniotic membrane and foetal skin. In the second half of pregnancy, it also contains foetal respiratory secretions, urine and excrement [5].

The AF is composed of water and electrolytes, lipids, proteins and hormones, vernix caseosa, lanugo hair and meconium and cells [6]. The cells present in the AF include a heterogeneous cell population with different morphologies, in vitro characteristics and in vivo potential. They are usually derived from the embryo, especially the amniotic membrane, respiratory, intestinal and urinary tracts. AF-derived cells gradually increase with gestational age unless a pathological condition alters cellular turnover. For example, cell counts are low in intrauterine death and urogenital atresia, whereas they are increased in anencephaly and spina bifida [7].

Besides the changing cell counts, the AF also contains a number of cells that vary in proportion according to gestational age. These cells were initially classified into amniocytes (60.8%), epithelioid (33.7%) and fibroblastic (5.5%) cells [8]. Recently, amniotic fluid stem cells (AFSCs) and amniotic fluid mesenchymal stem cells (AFMSCs) have also been isolated from AF. These are selectively cultured from the AF-derived cells using different selection processes and specific growth conditions.

Amniotic Fluid Stem Cells

AF may contain undifferentiated cells proved by the demonstration of the expression of skeletal muscle proteins when cells were cultured in the supernatant of rhabdomyosarcoma cell lines [9]. It was calculated that amniotic fluid-derived cells can differentiate into osteocytes, adipocytes and fibroblasts.

De Coppi et al. [10] used CD117 (a type III tyrosine kinase receptor for stem cell factor) as a method to select the undifferentiated population from the AF, via either magnetic or fluorescent-activated cell sorting (MACS and FACS, respectively).

The CD117-expressing (CD117þ) subpopulation (AFSCs) comprises 1% of cells and proliferates rapidly with clonal lines having a doubling time of 36 h [10]. These cells have potential to differentiate into all three germ layers.

The AFSCs may have a pluripotent potential similar to Embryonic Stem Cells (ESCs) or induced pluripotent stem cells (iPS) [11]. The pluripotent cells are defined by the ability to (1) be cultured indefinitely in an undifferentiated state, whilst remaining diploid with a normal karyotype; (2) form clonal lines; (3) differentiate towards all three germ layers in vitro; (4) form teratomas in vivo; and (5) differentiate into all three germ layers when injected into a blastocyst.

AFSCs can be reprogrammed to pluripotency without any genetic manipulation.

There is a great potential to use AFSCs for clinical purpose, especially because of their differentiation capabilities, in vitro culture characteristics and the lack of tumorigenic potential and ethical concerns. In addition, if pluripotency were needed, AFSCs could be efficiently reprogrammed to generate iPSCs. Finally, as AFSCs are derived from the foetus, they could be used as an autologous stem cell source for pre- and postnatal regenerative medicine applications.

Applications of Regenerative Medicine

Cardiovascular System

AFSCs cultured in cardiomyocyte induction media or in co-culture with cardiomyocytes demonstrated expression of proteins specific for cardiomyocytes (atrial natriuretic peptide and a-myosin heavy chain), endothelial (CD31, CD144) and smooth muscle cells (a-smooth muscle actin).

The left ventricular ejection fraction improved in animals who had ischaemia or perfusion injury (IR) that received the AFSC injection, as quantified using magnetic resonance imaging (MRI), suggesting a paracrine therapeutic effect [12]. Intravascular injection of human AFSCs showed a cardioprotective effect, improved cell survival and decreased the infarct size from 54% to around 40%.

Following intravascular injection, AFSCs engrafted in the lungs, heart and skeletal muscle, reducing the levels of brain natriuretic peptide (BNP), a surrogate marker for heart failure, and pro-inflammatory cytokines. AFSCs differentiated into endothelial and vascular cells forming micro-vessels, capillaries and small arteries [13].

In vitro, the AFSC displayed multi-lineage haematopoietic potential, demonstrated by the formation of erythroid, myeloid and lymphoid cells (haematopoietic lineages). In vivo, cells belonging to all haematopoietic lineages were found after primary and secondary transplantation of murine AFSCs into immunocompromised hosts, thus demonstrating the long-term haematopoietic capacity of these cells.

IUT (Intrauterine transfusion) has been proposed as an exciting application especially for the inherited haematological disorders (e.g. thalassemia and sickle cell disease) before birth management [14].

AFSCs are of foetal origin and are able to compete better against host cells. Because of the tolerogenic properties of the placenta, AFSCs are non-immunogenic to the foetus at any gestational age and also do not result in maternal immunization.

Gastrointestinal System

The effect of AFSCs was seen in a rat model of necrotizing enterocolitis (NEC) that involved hypoxia. NEC rats treated with AFSCs showed significantly higher survival at 7 days and had an improved NEC clinical status at 96 h.

Musculoskeletal System

AFSCs have osteogenic potential if they are cultured in a medium containing dexamethasone, b glycerophosphate and ascorbic acid-2-phosphate. After seeding in a collagen scaffold, they were implanted subcutaneously in mice. At 18 weeks, computed tomography (CT) revealed mineralized tissues and blocks of bone-like material [10]. Sun et al. studied osteogenic differentiation of human amniotic fluid stem cells (hAFSCs) by using bone morphogenetic protein-7 (rhBMP-7) and seeding on nanofibrous scaffolds, evidenced by alkaline phosphatase (ALP) activity, calcium content, von Kossa staining and the expression of osteogenic genes. Implantation into the subcutaneous space formed bone in 8 weeks with positive von Kossa staining and a radioopaque profile on X-ray [15].

The functional and stable long-term integration of AFS cells into the skeletal muscle of HSA-Cre SmnF7/F7 mutant mice, with a condition which closely replicate the clinical features of human muscular dystrophy [16]. Approximately, 25,000 freshly isolated AFSCs were directly injected into the tail vein of each animal without previous expansion in culture. This improved survival rate by 75% and restored muscle phenotype in comparison to untreated animals.

There is potential of AFSCs (1) to differentiate into the myogenic lineage; (2) to participate in muscle regeneration with muscle injury; and (3) to engraft the muscle stem cell niche. Hence, AFSCs is a promising therapeutic procedure for musculoskeletal and muscle degenerative diseases.

Nervous System

Human AFSC were injected in the brain of twitcher mice (model of Krabbe globoid leucodystrophy, associated with progressive oligodendrocyte and neuronal loss). Human AFSCs engrafted into the lateral cerebral ventricles and survived for up to 2 months. It also demonstrated that AFSC uptake was variable, with 70% of AFSCs surviving in the brain of twitcher mice, whereas only 30% of AFSCs survive in the brain of normal mice [10].

Respiratory System

AFSCs have been shown to have regenerative properties in the lungs, by differentiating according to the type of lung injury. AFSCs injected intravascularly into mice subjected to hyperoxia-induced pulmonary injury, migrate to the lung and expressed regenerating ability.

AFSCs have also been used in lung hypoplasia, especially in patients with congenital diaphragmatic hernia or prematurity. Early prenatal administration of AFSCs improves lung growth, bronchial motility and innervation [17].

Skin

Several million people worldwide suffer from severe burns each year, and about 10% of burn patients die due to deficiency of a suitable treatment. At present, skin repair

methods used to treat burns include autologous skin grafting, allogeneic skin grafting [18], and heterologous grafting. Problems with these methods include a shortage of donor skin, immune rejection and the spread of infection [19]. Therefore, there is an urgent need for a high-quality skin substitute for transplantation therapy. Human embryonic stem cells (hESCs) may offer a new approach to the treatment of skin injuries [20].

Foetal-derived stem cells represent a therapeutic possibility and can be obtained from human amniotic fluid using backup cells from amniocentesis specimens that would otherwise be discarded [21]. These human amniotic fluid stem (hAFS) cells express both embryonic and adult stem cell markers and can be induced to differentiate into cell types derived from different germ layers, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages [21].

B7H4 expression on hAFS cells is a key element for moderating inflammation and promoting wound repair. This finding is useful for future clinical applications of hAFS cells. It is believed that hAFS cells can provide a new source of skin tissue engineering. The hAFS cells can be used as a therapeutic option for worldwide shortage of donor skin tissue as hAFS cells can differentiate into keratinocyte precursors in vitro. These cells can also regenerate a pluristratified epithelium in tissue culture.

Urinary System

The evidence of a nephrogenic potential of AFSCs arises by experiments involving the ex vivo growth of murine embryonic kidneys that were injected with labelled AFSCs. The AFSCs were also shown to contribute to a number of components of the developing kidneys, such as the renal vesicle, S- and C-shape bodies. Besides, the extracellular matrix (ECM) and surrounding cells induced renal differentiation, with the AFSCs [22].

In a mouse model of acute tubular necrosis (ATN) caused by glycerol injection, AFSCs were injected intrarenally. This decreased creatinine and blood urea nitrogen (BUN) levels and reduced the number of damaged tubules, and increased proliferation of tubular epithelial cells. Besides, AFSCs injected during the acute phase of ATN (between 48 and 72 h) had no effect on creatinine and BUN levels, whereas AFSCs injected into the kidneys on the same day of glycerol injection resulted in no observed peaks in creatinine or BUN.

Early injection of AFSCs delayed interstitial fibrosis and progression of glomerular sclerosis, and prolonged animal survival.

Amniotic Fluid Mesenchymal Stem Cells

MSCs are pluripotent cells with a mesoderm potential, capable of differentiation into adipogenic, chondrogenic and osteogenic cell lineages.

MSCs derived from the AF (AFMSCs) have also been isolated and selected by the culture methodology.

AFMSCs in culture grow rapidly with an average doubling time of 1.6 days, which compares very well with that of BMMSCs (average doubling time 3.8 days). AFMSCs have a normal karyotype, up to 30 passages.

Applications of Regenerative Medicine

Cardiovascular System

MSCs have been used in an attempt to improve cardiac function with MSC therapy following an ischaemic injury.

Musculoskeletal System

AFMSCs were seeded on natural scaffolds and transplanted to replace a surgically created diaphragmatic defect in newborn lambs, as in vivo model of congenital diaphragmatic hernia (CDH).

AFMSCs were also assessed for their osteogenic potential in a number of in vivo models relating to surgically created osseous defects.

Nervous System

AFMSCs have also been used successfully against ischemic injury of the brain. The uses of AFMSCs were assessed for nerve injuries and the other conditions in which this neurogenic potential of AFMSC may be enhanced. A 5-mm defect was created in the sciatic nerve of rats and replaced the gap with gauze and fibrin glue with or without (control) embedded AFMSCs. The foot movement, muscle action potential, conduction latencies and axons that spanned more than half of the nerve gap were significantly better in the group with the AFMSCs, suggesting a neurotrophic effect on the Schwann cells [23].

The AFMSCs were injected intravascularly in the animals who had crush injury of nerves, it was found that the distribution of cells were time dependent. The cell migration to the nerve improved electrophysiological function, nerve myelination and expression of neurotropic factors [24].

Respiratory System

AFMSCs were seeded on synthetic scaffolds and incubated in chondrogenic medium for 24–30 weeks. Scaffolds seeded with AFMSCs were applied on partial or circumferential tracheal defects in foetal lambs, and that resulted in the presence of pseudostratified columnar epithelium present at birth, although moderate stenosis was present in all constructs.

Urinary System

The intravenous injection of human amniotic fluid stem cells into nonimmune-competent mice with glycerol-induced acute kidney injury showed rapid normalization of renal function compared with injection of mesenchymal stem cells. Both stem cell types showed enhanced tubular cell proliferation and reduced apoptosis. Mesenchymal stem cells were more efficient in inducing proliferation than amniotic fluid-derived stem cells, which, in contrast, were more antiapoptotic.

Summary

The AF is an under-utilized source of stem cells, with therapeutic potential in the field of regenerative medicine. Whilst a recent study shows that AF is a heterogeneous cell source, with high donor variation [25], the majority of the data available demonstrates that stem cells from the AF can be isolated and expanded easily, and have the ability to differentiate into a various cell types without the risk of tumorigenesis. Both amniotic fluid stem cells (AFSC) and amniotic fluid mesenchymal stem cells (AFMSC) hold great promise in the future of regenerative medicine.

References

1. Elliott MJ, De CP, Speggiorin S, et al. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year followup study. Lancet. 2012;380(9846):994–1000.
2. Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. Regen Med. 2010;1:121–43.
3. Zani A, Cananzi M, Fascetti-Leon F, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut. 2014;2:300–9.
4. Calvin SE, Oyen ML. Microstructure and mechanics of the chorioamnion membrane with an emphasis on fracture properties. Ann N Y Acad Sci. 2007;1101:166–85.
5. Lotgering FK, Wallenburg HC. Mechanisms of production and clearance of amniotic fluid. Semin Perinatol. 1986;10(2):94e102.
6. Cananzi M, Atala A, De CP. Stem cells derived from amniotic fluid: new potentials in regenerative medicine. Reprod Biomed Online. 2009;18(Suppl. 1):17–27.
7. Gosden C, Brock DJ. Combined use of alphafetoprotein and amniotic fluid cell morphology in early prenatal diagnosis of fetal abnormalities. J Med Genet. 1978;15(4):262–70.
8. Hoehn H, Bryant EM, Karp LE, et al. Cultivated cells from diagnostic amniocentesis in second trimester pregnancies. II. Cytogenetic parameters as functions of clonal type and preparative technique. Clin Genet. 1975;7(1):29–36.
9. Streubel B, Martucci-Ivessa G, Fleck T, et al. In vitro transformation of amniotic cells to muscle cells—background and outlook. Wien Med Wochenschr. 1996;146(9–10):216–7.
10. De CP, Bartsch G Jr, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol. 2007;25(1):100–6.
11. Parolini O, Soncini M, Evangelista M, et al. Amniotic membrane and amniotic fluid-derived cells: potential tools for regenerative medicine? Regen Med. 2009;4(2):275–91.
12. Bollini S, Cheung KK, Riegler J, et al. Amniotic fluid stem cells are cardioprotective following acute myocardial infarction. Stem Cells Dev. 2011;20(11):1985–94.
13. Angelini A, Castellani C, Ravara B, et al. Stem-cell therapy in an experimental model of pulmonary hypertension and right heart failure: role of paracrine and neurohormonal milieu in the remodeling process. J Heart Lung Transplant. 2011;30(11):1281–93.
14. Loukogeorgakis SP, Flake AW. In utero stem cell and gene therapy: current status and future perspectives. Eur J Pediatr Surg. 2014;24(3):237–45.
15. Sun H, Feng K, Hu J, et al. Osteogenic differentiation of human amniotic fluid-derived stem cells induced by bone morphogenetic protein-7 and enhanced by nanofibrous scaffolds. Biomaterials. 2010;31(6):1133–9.
16. Piccoli M, Franzin C, Bertin E, et al. Amniotic fluid stem cells restore the muscle cell niche in a HSA-Cre, Smn(F7/F7) mouse model. Stem Cells. 2012;30(8):1675–84.
17. Pederiva F, Ghionzoli M, Pierro A, et al. Amniotic fluid stem cells rescue both in vitro and in vivo growth, innervation, and motility in nitrofen-exposed hypoplastic rat lungs through paracrine effects. Cell Transplant. 2013;22(9):1683–94.
18. Metcalfe AD, Ferguson MW. Bioengineering skin using mechanisms of regeneration and repair. Biomaterials. 2007;28:5100–13.
19. Alsbjorn B. In search of an ideal skin substitute. Scand J Plast Reconstr Surg. 1984;18:127–33.
20. Odorico JS, Kaufman DS, Thomson JA. Multilineage differentiation from human embryonic stem cell lines. Stem Cells. 2001;19:193–204.
21. Tsai MS, Lee JL, Chang YJ, et al. Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. Hum Reprod. 2004;19:1450–6.
22. Perin L, Giuliani S, Jin D, et al. Renal differentiation of amniotic fluid stem cells. Cell Prolif. 2007;40(6):936–48.
23. Pan HC, Yang DY, Chiu YT, et al. Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell. J Clin Neurosci. 2006;13(5):570–5.
24. Pan HC, Cheng FC, Chen CJ, 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.
25. Ekblad A, Qian H, Westgren M, et al. Amniotic fluid—a source for clinical therapeutics in the newborn? Stem Cells Dev. 2015;24(12):1405–14.