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HUMAN AMNIOTIC FLUID CELLS AND THEIR FUTURE PERSPECTIVES

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ABSTRACT

Amniotic fluid holds many important roles in the development of fetus such as supporting the growth of the fetus as well as protecting it from any distress or infection. The fluid is also a vital source of fetal cells used in prenatal diagnosis, but it will be discarded if such procedure is not conducted or requested by the patient. Recently there are many reports on the cells present in amniotic fluid, as the population of cells is heterogenous with various types of cells, including differentiated and undifferentiated cells. These cells are believed to hold various potential, including in therapeutic applications, as they exist with the fetus since the embryonic stage. In this review, we have summarized some of the important aspects of amniotic fluid and update on the cells present in amniotic fluid, together with its future perspective. In a nutshell, amniotic fluid is a very valuable tool that could save lives in the future, especially through regenerative medicine.

Introduction

Amniotic fluid (AF) is the clear, yellowish fluid that surrounds fetus during pregnancy [1]. The fluid is very important in the survival of fetus as it supports the fetus movement and growth by providing space and suitable environment, and protects the fetus by cushioning it from fetal trauma and bacterial infection [2,3].

Amniotic fluid starts to appear as early as the 2nd week of gestation, as a result of the flow of fluid from fetal lung and bladder [2] and it gradually expands between the 8th and 10th day after fertilization [4]. Water is the biggest component of AF (98-99%), followed by solids (1-2%) [5]. AF also consists of inorganic substances, which is part of the solids, such as proteins, glucose, enzymes, hormones, lipid and suspended materials like vernix and meconium [5,6].

In clinical setting, AF is the source of fetal cells for fetus genetic analysis and gender determination of the fetus during pregnancy [7]. Interestingly, various types of fetal cells in AF have been discovered, making the fluid more exciting to be explored. This review aims to update on the cells found in AF and their future perspective.

Cell population within Amniotic fluid

Amniotic fluid consists of heterogenous population of undifferentiated and mature cells that begins to appear at about 14 weeks gestation [1, 6, 8]. Heterogeneity of AF cells may be explained by the direct contact of AF with the fetus as well as the diverse origin of the cells, mainly from the three primary germ layers of amnion and embryonic tissues [9, 1, 10].

These cells can be cultured from as little as 2 to 3ml of AF, ideally from 15 up to 22 weeks of gestation as high successful

rate of AF cell cultures were reported during this period [11, 6, 5]. Furthermore, as gestation period develops, the number of cells in AF increases but the viable cells decreases [12, 13, 14]. In addition, pregnancy with female fetuses was discovered to contain higher cell count in the final trimester, compared to pregnancy with male fetuses, due to the presence of vaginal cells [6].Upon culturing AF cells *in vitro*, the average time for cell attachment is 3-7 days [11, 6].

Based on marker expression of the cell lineages, more of mesodermal and endodermal cells are present during early gestational period while equal amount of ectodermal cells are detected in AF at both late and early gestational periods [27, 9]. This may suggests that the type of cells present in AF follows the development of human embryo during gestation, in addition more cells with organ specific markers were

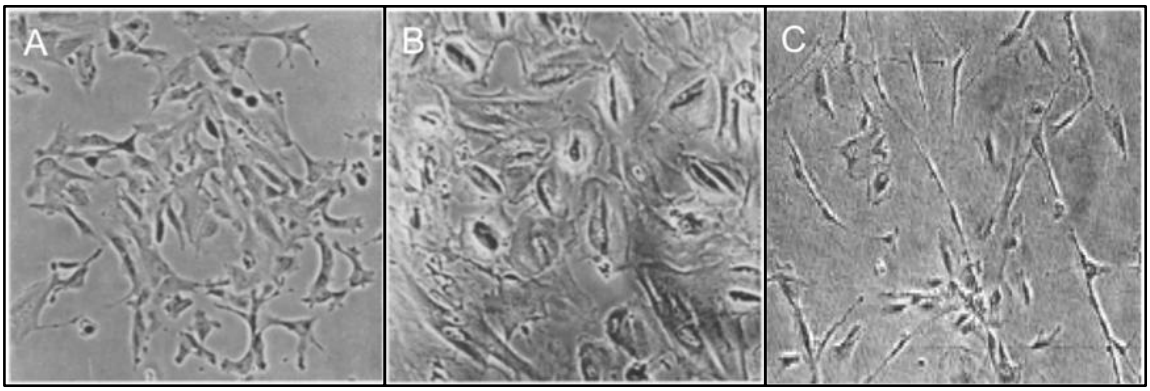

Fig.1 Morphology of different types of cells in AF. AF-type cells show a mixture of fibroblast- and epithelial-like cells (A). E-type cells show epithelial-like cells (B) and F-type show spindle-shaped cells (C). [15, 16].

Based on the cell morphology, AF cells can be categorized into 3 types; amniotic fluid (AF)-type, epitheloid (E)-type and fibroblastic (F)-type cells [15]. Figure 1 illustrates the comparative morphologies of the cells, with distinct morphology between E-type cells and F-type cells. AF-type cells are specific cells in AF with subtle difference from F-type cells, classified by a group of researchers in USA back in 1974 [16].

From the literature, there are different opinions regarding the most dominant cells in AF cell culture. Although AF-type cells have been reported to be dominant (60-70%), followed by E-type (20-30%) and F-type (<10%) cells [17, 16], other literature reported AF to consist more of E-type cells, whereas F-type cells were detected occasionally [1, 18, 5]. Conversely, F-type cells were also reported to be dominant as they are easier to subculture, more selective and have highest growth potential [16, 19, 20]. Nonetheless, AF-type cells and some of F-type cells are thought to have epithelial or endothelial in origin [18, 21]. Different source of each type of cells have also been described; AF-type cells are from fetal membranes and trophoblast, whereas E-type cells are from fetal skin and urine and F-type cells are from dermal fibroblasts and fibrous connective tissues [22, 6, 23]. In addition, E-type cells obtained from cesarean section were reported to express neuronal and glial markers and able to differentiate into neuron-like cells [24, 25, 26].

detected in late gestation, where the organs of the fetus has matured and fetus has formed completely [27].

Recent discovery also mentioned that since fetal urine constitutes most of the AF volume, kidney progenitor cells should be the major cells present in AF and in fact, the expression of renal markers increase by the end of 17 weeks gestation [27].

Stem Cells in Amniotic Fluid

Although AF cells were reported to have limited proliferative capacity and are terminally differentiated cells [28, 29], telomerase activity was detected in both cultured and uncultured human AF cells from 14 weeks gestation, suggesting that cells with high proliferative capacity exist in AF [30]. Furthermore, expression of Oct4 (critical marker for pluripotency) was also discovered, indicating the presence of pluripotent subpopulation of cells in AF - stem cells [31].

Subsequently, more findings on stem cell population in AF were reported [32, 33, 34]. Populations of stem cells in AF are based on their potency, either multipotent or pluripotent. There are 2 types of multipotent AF cells discovered; the amniotic fluid c-Kit+, Lin- (AFKL) cells and amniotic fluid mesenchymal stem cells (AF-MSCs) [35, 33]. AFKL are multipotent AF cells having multilineage hematopoetic potential *in vitro* and express CD45 [35] while AF-MSCs are multipotent towards mesenchymal lineage [33]. So far there is

only 1 report on AFKL cells, thus further study on these cells is essential.

Amniotic fluid mesenchymal stem cells (AF-MSCs) are more abundant in AF and easier to culture, based on the 100% success rate of the cultured cells [38, 39]. They can be obtained from mid- and full-term pregnancies without any selection step, and cultured in serum-rich condition, without feeder layers and supplemented with FGF (5ng/ml) [33]. Alternatively, AF-MSCs can also be cultured without animal serum and still retain their properties [40]. Although these cells have fibroblast-like and spindle-shaped morphology, similar to other types of adult MSCs (Figure 2), AF-MSCs was reported to have the highest proliferation capacity [36, 37].

On the other hand, specific population of amniotic fluid stem (AFS) cells represents only 1% of AF and can be isolated from mid-trimester AF through immunoselection of AF cells expressing c-Kit, a stem cell factor receptor involved in embryogenesis, carcinogenesis and hematopoiesis, indicating that it can identify stem cell population within different organs [9, 32]. Unlike AF-MSCs, to date, AFS cells could only be found in mid-trimester pregnancy as the cells disappeared slowly at 19-20 weeks gestation [11, 27, 32].

AFS cells are positive for ES cell marker (SSEA-4), mesenchymal stem cell markers (CD90, CD73, CD105), several adhesion molecules (CD29, CD44) and antigens of major histocompatibility complex I (MHCI). However, they are negative for endothelial and haematopoietic markers (CD133, CD14, CD31, CD35, CD45) and antigens of major histocompatibility complex II (MHC-II) [32].

Most importantly, AFS cells also express the key molecular markers of pluripotency, specifically Oct-4, Sox2 and Nanog, suggesting that AF could be the new source of pluripotent stem cells [11, 31]. Furthermore, Oct-4 positive cells in amniotic fluid are clonogenic and in actively dividing state as they express the marker for cell cycle proliferation, cyclinA [43].

Essentially, AFS cells have similar potency as embryonic stem (ES) cells as they are able to give rise to tissue derivatives of the three primary germ layers, though AFS cells are more advantageous as they are not bound to ethical restrictions and they are non-tumorigenic [32, 33, 44]. In addition, the ability of AFS cells to form embryoid bodies upon spontaneous differentiation has validated the pluripotentiality of AFS cells [44].

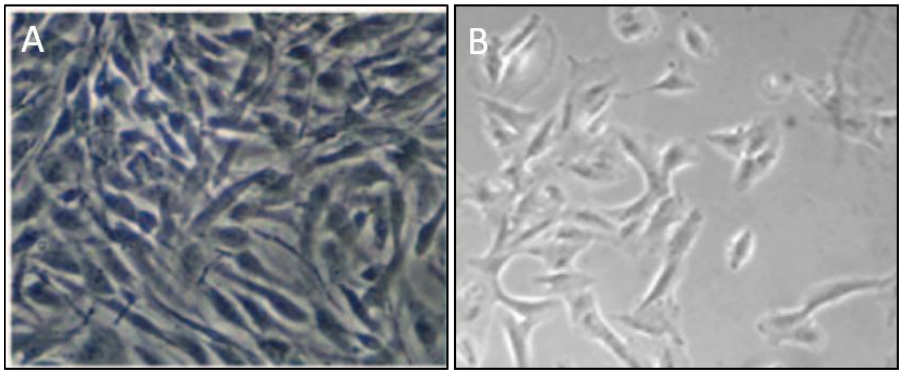

Fig.2 Stem cells in amniotic fluid. (A) Amniotic fluid mesenchymal stem cells (AF-MSCs), (B) Amniotic fluid stem cells (AFSCs) [38, 41].

AFS cells have a mixture of fibroblast-like to oval-round shape morphology (Figure 2). Although the cells exhibit slow proliferation in the initial stage, they will eventually adhere to the culture flask after a week [42] and propagate rapidly in culture with doubling time of 36h [32, 41]. They have high clonogenicity and self-renewal capacity, whereby they can be expanded for over 250 doublings without any detectable loss of chromosomal telomere length and retain normal, diploid karyotype [32]. AFS cells are confirmed of fetal origin by obtaining AF samples from pregnancies with male fetus only which is shown by male karyotype analysis [9]. AFS cells also did not form tumors following transplantation into severe combined immunodefficient (SCID) mice [32].

Future Perspective

The presence of various mature, differentiated cells in AF makes AF an excellent source for tissue engineering. In fact, this notion has started in 2001 [37] and followed by a group that focus on renal cells from AF for transplantation [27]. Most importantly, the discovery of stem cells in AF, specifically AFS cells, has elevated the potential of AF as AFS cells signify a novel class of pluripotent stem cells with intermediate characteristics of ES cells and adult stem cells [45, 28].

Besides of being easy to maintain, proliferate and differentiate [46], AFS cells are safer and ethical to use in clinical

applications because they are non-tumorigenic and more accessible, causing harm neither to the mother nor the fetus [38, 32]. Furthermore, AFS cells would be an ideal candidate for autologous transplantation as they lack of MHC Class II antigen, therefore non-immunogenic [47]. These advantages have opened interesting future directions for AF both in *in vitro* and *in vivo.*

AFS cells are valuable candidate for applications in tissue regeneration mechanism as they are easily infected with retrovirus and lentivirus, following great capacity to express reporter gene marker proteins such as Green Fluorescent Protein (GFP), luciferase and Lac-Z in *in vitro*, as well as *in vivo* [9]. Fundamental studies on stem cells and drug screening [42] could also benefit from AFS cells, as these cells did not require any modification to undergo differentiation [48]. In addition, AF cells exhibit high reprogramming efficiency as they were reported to form induced pluripotent stem (iPS) cells more rapidly (in 6 days) compared to other cells (several weeks) [49]. Therefore, AF cells might be useful in generating disease specific stem cell lines.

In complement to *in vitro*, *in vivo* studies emphasize the true clinical potential of AFS cells. AFS cells *in vivo* are able to supply various differentiated cells such as kidney, cartilage, lung and bone, useful in repair or regeneration purposes through tissue engineering [42, 46]. Therefore they hold great potential in cell therapies and regenerative medicine. In fact, these cells are a natural target and a good candidate for gene therapy as the cells are highly proliferative and less differentiated [41, 50].

Furthermore, initial results of AFS cells in preclinical trials are promising, such as in kidney, bone and lung preclinical studies. AFS cells has been shown to express early kidney molecular markers: Glial cell-derived neurotrophic factor (GDNF), ZO-1 protein and claudin when injected into mouse kidney [48] and have successfully been transplanted into a nude rat when the cells were pre-differentiated into bone forming cells, resulting in an increasing of bone formation [51]. Similarly, AFS cells in lung preclinical studies also have been found to express specific alveolar and epithelial markers and demonstrate a strong tissue engraftment when systemically administered into injured lung [52]. These preliminary results will certainly lead to interesting developments of AFS cells in the future [46].

Conclusion

In summary, the application of AF has emerged from being a diagnostic tool to an important resource for regenerative medicine. The wide spectrum differentiation capacity of AFS cells has made AF a promising and a powerful tool in fundamental and applied research. Hence, more studies are essential in exploring the differentiation potential of these cells into functional specific cell types for bedside applications.

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