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LITERATURE REVIEW

Isolation of Amniotic Fluid Mesenchymal Stem Cells (AF-MSCs) Obtained From Caesarean Sections

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Abstract

Amniotic fluid is a liquid that fills the amniotic cavity which has defense and nutritional functions in fetal development. Human aterm amniotic fluid can be an ideal alternative as a source of mesenchymal stem cells, originating from the neonate. Preclinical studies of second and third trimester amnion fluid cells confirmed the number of potential donors from this wasted material. In several studies, AF-MSCs express mesenchymal markers such as CD90, CD73 (SH3, SH), CD105 (SH2), CD29, CD166, CD49e, CD58 and CD44 (MHC class I). These cells also express HLA-ABC antigens, CD 34, CD 45 which are hematopoietic markers, and endothelial CD31 markers. There is no expression of CD10, CD11b, CD14, CD34, CD117, EMA and HLA-DR, DP, DQ antigens. Most of AF-MSCs have pluripotent properties which are characterized by the discovery of octamer binding protein 3/4 (Oct-3/4), transcription factors Nanog (Nanog), and stage-specific embryonic antigen 4 (SSEA-4) on RT-PCR examination. From this study, 8 million cells was isolated. These cells will be used for research on pelvic organ prolapse therapy by using AF-MSCs. AF-MSCs isolation totally takes 6 weeks. From 1 flask, 2 million of stem cells was obtained.

***Keywords:** amniotic fluid, AF-MSCs*

INTRODUCTION

Amniotic fluid is a liquid that fills the amniotic cavity which has defense and nutritional functions in fetal development. The volume and composition of amniotic fluid can be changed according to the age of pregnancy. At the beginning of fetal development, amniotic fluid is produced by maternal plasma. In 8 weeks gestation, the kidneys begin to produce fluids that significantly add amniotic fluid. Recent findings on stem cell sources show that amniotic fluid contains novel stem cells (AFS = Amniotic Fluid Stem Cell), characterized by c-kit expression (stem- cell factor receptor), illustrating that AFS can be used in regenerative medicine.[1]

There are a heterogeneous population of the three developmental plates in amniotic fluid. In this tissue, cells are found from the inner layer of the amniotic membrane or from developing fetal cells. Cells in amniotic fluid are grouped based on morphology, growth and biochemical characteristics. Epitheloid cells (E-type) are cuboid to columnar cells that originate from the skin and urine of the fetus, amniotic fluid cells (AF-type) from fetal membranes (AF-type) and fibroblastic (F-type) cells originating from connective tissue fibrous.

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Immunophenotypes showing amniotic fluid stem cells is dominated by mesenchymal stem cells known as Amniotic Fluid-Derived Mesenchymal Stem Cells (AF-MSCs).[2]

Mesenchymal Stem Cells (MSCs) are multipotent and self renewal cells. In the study of Asl et al. comparison between mesenchymal cells originating from human amnion and mesenchymal cells originating from adipose cells. In this study, a higher proliferative power was obtained in mesenchymal cells originating from human amnion compared to those derived from human adipose cells. In the study of Gafaar et al. a comparison is made between MSC on amnion, endometrium and ovary. All three have almost the same molecular structure, but MSC derived from amniotic fluid has a lower immune response and higher transplant success in xenogenetic hosts.[3,4]

Human aterm amniotic fluid can be an ideal alternative as a source of mesenchymal stem cells, originating from the neonate. Preclinical studies of second and third trimester amnion fluid cells confirmed the number of potential donors from this wasted material. Human amniotic fluid stem cells (hAFSCs) can be obtained noninvasively. hAFSCs have proliferative rates, induce immunological tolerance, describe embryonic stem cell properties and can differentiate into three developmental plates and several cell lineages, such as adipocytes, osteoblasts, chondrocytes, kidney cells, hepatocytes and cardiomyocytes. This characteristic suggests that hAFSC can be an ideal source of application for stem cell therapy.[5,6]

METHODS

Amniotic Fluid-Mesenchymal Stem Cell

Amniotic fluid has an essential function in fetal development, nutrition and defense. The volume and composition of amniotic fluid already changed according to the age of pregnancy. Amniotic fluid is also used for screening of genetic disorders, embryos, infections and congenital disorders. Besides that, amniotic fluid has some various of stem cells from developing fetuses which can be a source of stem cell development.[1,7]

Amniotic fluid is a promising source of stem cells. Amniotic fluid is found in many types of stem cells, from primordial cells that are pluripotent to specific organ progenitor cells and adult cells. In addition, hematopoesis progenitor cells, genetic markers of specific tissues such as the brain, heart and pancreas were also found. Recently, amniotic fluid has become a source of mesenchymal stem cells for therapeutic therapy with minimal risk of tumor formation.[1,8]

Tsai et al. (2004) in their study succeeded in isolating amniotic fluid- derived mesenchymal stem cell (AF-MSC) in the second trimester of pregnancy. Isolation was obtained without disturbing the karyotype process of the fetus using the novel two-stage culture protocol method. Cells obtained from this process can develop into several cell types in vitro.[9]

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Advantages of AF-MSCs

Amniotic stem cells (ASC) can be extracted from the amniotic sac through the amniocentesis process, where this process can be carried out without injuring the developing fetus. This is very important to emphasize because of the negative stigma towards embryonic derived stem cells. Another study showed isolation of amniotic fluid through cesarean section labor by using a catheter.[5,10]

Amniotic fluid and ASC have many advantages related to medical use. First, fluid and amniotic cells have immunological features which means they do not cause a reaction even among donors and recipients who are totally unrelated. Second, amniotic fluid contains more stem cells than the adult bone marrow. ASC is like a hybrid between embryonic stem cells and adult stem cells in terms of plasticity and differentiation ability. Third, amniotic fluid contains hyaluronic acid, which helps lubrication and can be used in the treatment of joints. Fourth, amniotic fluid contains a complete complement of growth factors that stimulate stem cells to differentiate into many cell types. Tehn, amniotic fluid is naturally antimicrobial and can prevent and eliminate potential infectious agents.[10] In the comparison of AFMSCs with BMSC according to the same growth time (day 15), amniotic cells showed more cells than stem cells originating from the ACS bone marrow. It normally larger and more numerous than stem cells originating from the bone marrow, indicates AFMSCs grow and differentiate faster. This is needed for treatment because it shortens the patient's healing time, and can also reduce the amount of treatment needed.[10]

Isolation and characteristics of AF- MSCs

Amniotic fluid is generally obtained from diagnostic actions of amniocentesis and therapeutic amnioreduction. Although the chemical and cellular composition varies from each gestational age, MSC can be isolated at any time during pregnancy. There are several isolation techniques of AF-MSCs that are commonly used, namely multiple wells and coverslips.[11]

The multiple wells isolation method was obtained by inserting 40 ml of amniotic fluid into a 50 ml centrifugation tube. Amniotic fluid was centrifuged 500 x g for 15 minutes at room temperature. Supernatants were discarded and the pellet was resuspended at 6ml Dulbecco's modified Eagle's medium (DMEM) 20% FBS stock (Medum mesenkimal-20). Then, 1 ml of the suspension cell was applied to the collagen-coated 6-well culture plate and incubated for 48 hours. The medium is replaced by aspirating and replacing 1 ml of medium per plate. This function is to remove cells that are not attached. Then the medium is replaced every 3 days. On days 7 and 14, expansion was carried out on a plate that had not contaminted mesenchymal morphology. The medium was aspirated and washed by adding CMF- DPBS (Dulbecco's phosphate-buffered saline).

Then 0025% trypsin / 0.04% EDTA was added and incubated for 2-4 minutes. Suspension

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cell was transferred into a 15 ml centrifuge tube with the addition of 8 ml of the 20-mesenchymal medium and centrifuged 400 x g for 5 minutes at room temperature. The pellets are resuspended in 6 ml of the 20-mesenchymal medium. Suspension cells were taken as much as 1 ml and planted on culture platescontaining 9 ml of mesenchymal medium-20 to 80-90% confluent before being expanded.[11]

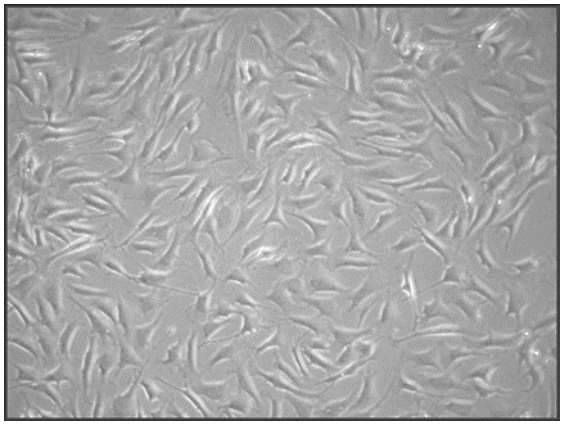

Figure 1 Homogeneous population of AF-MSCs after isolation (100x magnification)[11]

In the coverslips method, mechanical separation is obtained by placing a pellet on the cover glass of the object distributed on a 10 cm collagen- coated plate with cell density of 2-3 million cells in 150cm2. After 48 hours, the cover glass of the object was observed and selected cells containing mesenchymal cells were placed on a 30 cm2 cultureplate (containing the 20-mesenchymal medium). The medium is replaced every day following the steps of the multiple wells method.[11]

MSC develops according to the growing medium. In analyzing surface antigens with flow cytometry, polymerase chain reaction and staining of immunocytochemistry, AF-MSCs were expressed the same mesenchymal markers from other sources. In several studies, AF-MSCs express mesenchymal markers such as CD90, CD73 (SH3, SH), CD105 (SH2), CD29, CD166, CD49e, CD58 and CD44 (MHC class I). These cells also express HLA-ABC antigens, CD 34, CD 45 which are hematopoietic markers, and endothelial CD31 markers. There is no expression of CD10, CD11b, CD14, CD34, CD117, EMA and HLA-DR, DP, DQ antigens. Most of AF-MSCs have pluripotent properties which are characterized by the discovery of octamer binding protein 3/4 (Oct-3/4), transcription factors Nanog (Nanog), and stage-specific embryonic antigen 4 (SSEA-4) on RT-PCR examination.[2,9,12]

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Along with the development of stem cell research, a standard criteria for MSC is needed. The international study for cellular therapy recommends the minimum criteria for determining of MSC. These criteria include:[13,14]

1. Derived from the fetus, ≤ 1% maternal contamination
2. MSC has plastic-adherence properties.
3. Can develop to form *colony forming unit-fibroblast* (CFU-F).
4. Having specific antigen expression.
   Population of MSC can express CD105 (endoglin), CD73 (ecto 5 'Nucleotidase) and CD 90 (Thy-1) of ≥ 95% as measured by cytometry flow. There was a lack of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II expressions (≤2% positive).
5. MSC must be multipotent differentiated into osteoblasts, adipocytes, and chondroblasts under in vitro standard conditions.

DISCUSSION

Isolation of AF-MSCs

Amniotic fluid was isolated from aterm pregnant women (37-41 weeks' gestation) through elective caesarean section surgery by inserting a plastic catheter into the intact amniotic membrane, with the following procedure:[5]

1. Perforation of amniotic membrane using a soft plastic catheter is then inserted into the amniotic cavity to collect amniotic fluid. The catheter system is a developed prototype to take high volume of amniotic fluid.
2. Amniotic fluid samples are moved into a collection of drainage containers which can expand through a closed sterile tube with passive suction. Processing of cellular material from amniotic fluid is carried out in 2-4 hours.
3. Amniotic fluid is filtered using 100 um Cell strainer.
4. The filtered sample was centrifuged at 850 × g for 5 minutes.
5. The supernatant is removed and the pellet is resuspended by using a complete medium Agf with 850 xg speed for 20 minutes.
6. After being centrified, the supernatant is removed and the pellets are suspended in a complete medium. Once homogeneous, plant cells in a 24-well plate.
7. Do the alternation of the medium every 3-4 days. If it is 80-90% confluent, then do a cell subculture.

Colony forming unit-fibroblast assays

After 11-14 days of cell culture, the number of *colony forming unit-fibroblast* (CFU-F) was assessed microscopically by calculating spindle-shaped colonies that clearly resembled of

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fibroblasts, and did not include colonies with rounded epithelioid morphology. Colonies containing ≥40 cells were counted.[5] Amniotic fluid culture was carried out in the biomedical laboratory of Andalas University.

Flow Assays

Single cell suspensions of confluent cultures 3–5 in media 1 and 2 were prepared and stained with fluorescent labeled antibodies. Antibodies that is used include CD90, CD73, CD105, CD29, CD166, CD49e, CD58, CD44 (Roubelakis et al., 2012) For OCT4 intracellular staining, the cells is fixated (4% PFA) and stabilized (0.5% Triton X-100) before staining. The function of isotype antibodies are as controls. Quantitative analysis was carried out using FACSCantoII flow cytometer (BD) and FlowJo software.[5] The flow test is carried out in the IMERI laboratory of Indonesian University.

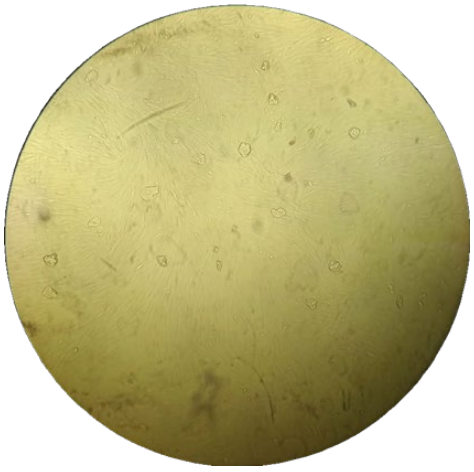

Figure 2. Amniotic fluid cells

From this study, we succeeded in getting 8 million cells which will be used for research on pelvic organ prolapse therapy by using AF-MSCs. The stem cells isolation totally takes 6 weeks. We got 2 million stem cell cells in 1 flask.

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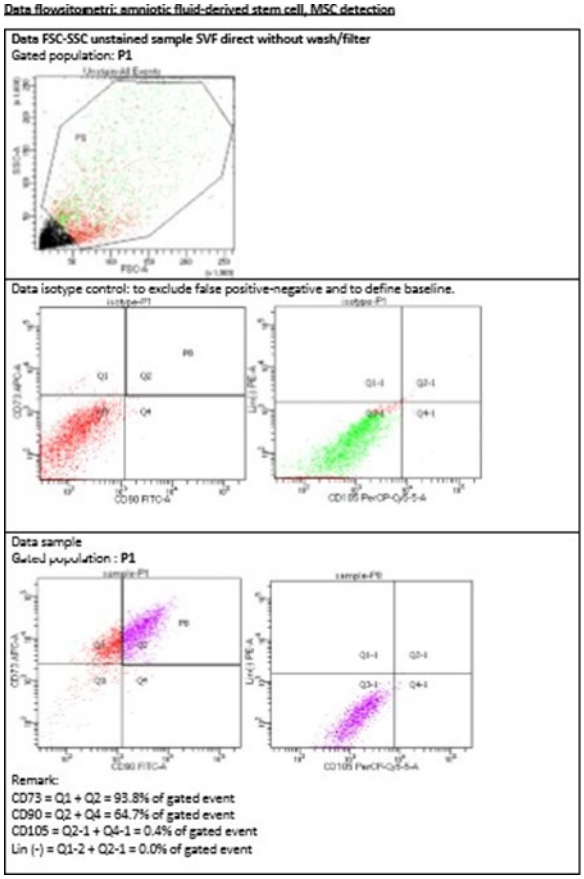

CONCLUSION

Amniotic fluid has an essential function in fetal development, nutrition and defense. The volume and composition of amniotic fluid already changed according to the age of pregnancy. Amniotic fluid is also used for screening of genetic disorders, embryos, infections and congenital disorders. Besides that, amniotic fluid has some various of stem cells from developing fetuses which canbe a source of stem cell development. Recent findings on stem cell sources show that amniotic fluid contains novel stem cells (AFS = Amniotic Fluid Stem Cell), characterized by c-kit expression (stem- cell factor receptor), illustrating that AFS can be used in regenerative medicine. Amniotic fluid is generally obtained from diagnostic actions of amniocentesis and therapeutic amnioreduction. Although the chemical and cellular composition varies from each gestational age, MSC can be isolated at any time during pregnancy. There are several isolation techniques of AF-MSCs that are commonly used, namely multiple wells and coverslips. From this study, we succeeded in getting 8 million cells which will be used for research on pelvic organ prolapse therapy by using AF- MSCs. The stem cells isolation totally takes 6 weeks. We got 2 million stem cell cells in 1 flask.

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