ORIGINAL ARTICLE

Medical Journal of Western Black Sea
Batı Karadeniz Tıp Dergisi

Med J West Black Sea 2025;9(2): 166-174
DOI: 10.29058/mjwbs.1586041

Isolation and Characterization of Mesenchymal Stem Cells Derived from Human Amniotic Membrane by Explant Technique

İnsan Amniyon Zarından Eksplant Teknikle Elde Edilen Mezenkimal Kök Hücrelerin İzolasyonu ve Karakterizasyonu

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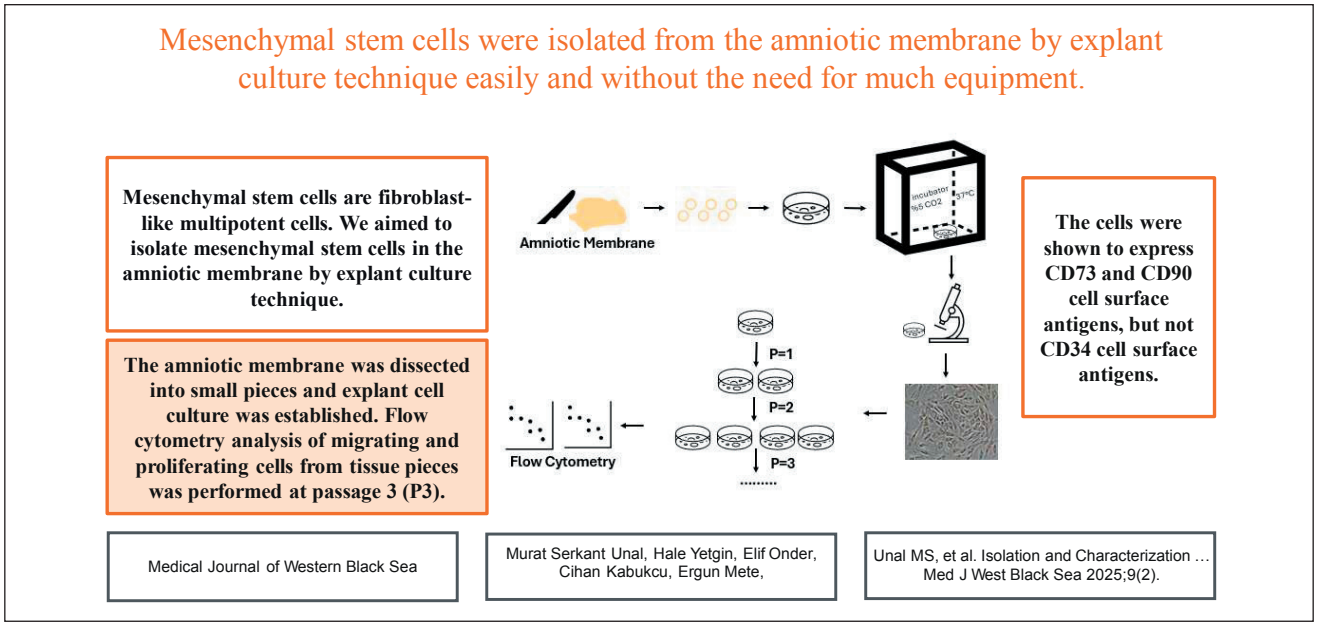
Cite this article as: *Ünal MS et al. Isolation and characterization of mesenchymal stem cells derived from human amniotic membrane by explant technique. Med J West Black Sea. 2025;9(2): 166-174.*

GRAPHICAL ABSTRACT



ABSTRACT

Aim: Mesenchymal stem cells (MSCs) are adult stem cell types. When examined by phase contrast microscopy, they appear spindle-shaped and fibroblast-like cell aggregates. Up to now, MSCs have been isolated from many tissues such as bone marrow, adipose tissue, cartilage, placenta, amniotic membrane and umbilical cord. Our aim in this study was to isolate mesenchymal stem cells from amniotic membrane by explant culture technique easily and without needing a large amount of equipment.

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Received: 19.11.2024 **Revision:** 04.08.2025 **Accepted:** 04.08.2025

Material and Methods: In the cell culture laboratory, amniotic membranes were dissected into small pieces and explant culture medium was created. Then, flow cytometry analysis was performed at passage 3 to determine the characterization of proliferating cells. Differentiation experiments were conducted to show the changes in adipogenic, chondogenic and osteogenic direction.

Results: In our study, mesenchymal stem cells were isolated from the human amniotic membrane by explant cell culture technique. Flow cytometry analysis showed that the cells expressed mesenchymal stem cell markers (CD73 and CD90) but not hematopoietic stem cell markers (CD34). In differentiation experiments, adipogenic, chondrogenic and osteogenic changes were observed.

Conclusion: After the widespread use of mesenchymal stem cells isolated from the umbilical cord as a valuable source of regenerative and reparative medicine, studies on the isolation of mesenchymal stem cells from the amniotic membrane have intensified.

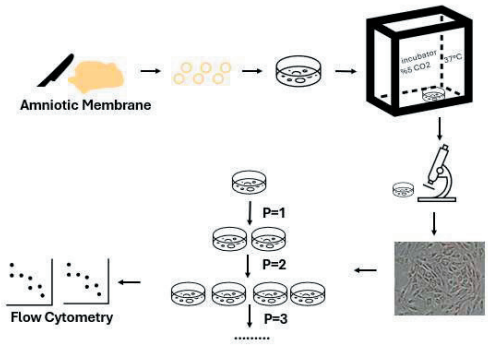
Keywords: Cell culture techniques, amnion, stem cells

GRAFİKSEL ÖZET

Amniyon zarından eksplant kültür tekniğiyle mezenkimal kök hücreler kolay bir şekilde ve fazla ekipmana ihtiyaç olmadan izole edilmiştir.

Mezenkimal kök hücreler fibroblast benzeri multipotent hücrelerdir. Amacımız amniyon zarında mezenkimal kök hücreleri eksplant kültür tekniğiyle izole etmektir.

Amniyon zarı küçük parçalara ayrılarak eksplant hücre kültürü oluşturuldu. Doku parçalarından göç eden ve prolifere olan hücrelerin 3. pasajda(P3) flow sitometri analizleri yapıldı.



Hücrelerin CD73 ve CD90 hücre yüzey antijenlerini eksprese ettikleri, CD34 hücre yüzey antijenlerini ise eksprese etmedikleri gösterilmiştir.

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Unal MS ve ark. İnsan Amniyon Zarından Eksplant … Med J West Black Sea 2025;9(2).

ÖZ

Amaç: Mezenkimal kök hücreler (MKH) erişkin kök hücre tipidir. Faz kontrast mikroskobu ile incelendiğinde iğ seklinde ve fibroblast benzeri hücre toplulukları olarak görülürler. Şimdiye kadar MKH'ler, kemik iliği, adipoz doku, kıkırdak, plasenta, amniyon zarı, göbek kordonu gibi birçok dokudan izole edilmişlerdir. Bu çalışmadaki amacımız amniyon zarından mezenkimal kök hücreleri eksplant kültür tekniğiyle kolay bir şekilde ve fazla miktarda ekipmana ihtiyaç olmadan izole etmektir.

Gereç ve Yöntemler: Hücre kültürü laboratuvarında amniyon zarları küçük parçalara ayrılarak eksplant hücre kültür ortamı oluşturuldu. Daha sonra prolifere olan hücrelerin karakterizasyonunu belirlemek amacıyla 3. pasajda flow sitometri analizleri yapıldı. Adipojenik, kondojenik ve osteojenik yöndeki değişimlerini göstermek için ise farklılaşma deneyleri yapıldı.

Bulgular: Çalışmamızda insan amniyon zarından mezenkimal kök hücreler eksplant hücre kültür tekniğiyle izole edilmişlerdir. Yapılan flow sitometri analizinde hücrelerin mezenkimal kök hücre belirteçlerini (CD73 ve CD90) eksprese ettiklerini hematopoetik kök hücre belirtecini (CD34) ise eksprese etmedikleri gösterilmiştir. Farklılaşma deneylerinde ise adipojenik, kondojenik ve osteojenik yönde değişim gösterdikleri belirlenmiştir.

Sonuç: Göbek kordonundan izole edilen mezenkimal kök hücrelerin rejeneratif ve reparatif tıpta değerli bir kaynak olarak yaygın bir şekilde kullanılmasından sonra amniyon zarından elde edilen mezenkimal kök hücreler üzerinde de çalışmalar yoğun bir şekilde devam etmektedir.

Anahtar Sözcükler: Hücre kültürü teknikleri, amniyon, kök hücreler

INTRODUCTION

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs), first described by Friedentstein and colleagues in 1974, are cells with a fibroblast-like appearance that can differentiate into osteocytes, chondrocytes, adipocytes, tenocytes and myocytes (1,2). MSCs were first isolated from bone marrow (3). Subsequently, they were isolated from different tissues including brain, periosteum, liver, skeletal muscle, adipose tissue and amniotic fluid.

The International Society for Cell Therapy (ISCT) has developed three criteria to define MSCs: 1) adhesion of the cells to a plastic surface in culture medium 2) differentiation into osteocytes, adipocytes and chondrocytes and 3) expression of markers such as CD105, CD73 and CD90 in more than 95% of the culture, absence of expression of markers such as CD34, CD45 in more than 95% of the culture (4).

Since MSCs do not have MHC class II antigens, they do not cause immune responses. However, there are some phenotypic differences between MSCs isolated from different tissues; for example, MSCs derived from adipose tissue express CD49d but do not express CD106, while those isolated from bone marrow are negative for CD49d and positive for CD106. These characteristics play a significant role in the potential therapeutic applications of MSCs.

When MSCs are examined microscopically, they are observed as spindle-shaped and fibroblast-like cell communities (5). MSCs have recently attracted interest in different clinical areas due to their potential therapeutic effects (2). Finding a safe and appropriate source of stem cells that can be used for treatment is crucial for regenerative medicine. Stem cells derived from gestational tissues have some advantages over other stem cells. These include easy access to gestational tissues, fewer ethical and legal barriers associated with their use, availability without the need for invasive procedures, and most importantly, they do not cause tumor formation. MSCs derived from the amniotic membrane(AM) have the capacity to differentiate into the three germ layers, have low immunogenicity and anti-inflammatory properties (6).

Mesenchymal Stem Cells Derived from Amniotic Membrane

Fetal membranes are divided into an outer layer (chorion) and an inner layer (amniotic membrane) that come into contact with maternal cells. The amnion surrounds the embryo/fetus as a thin membrane on the inner side of the placenta and borders the amniotic cavity filled with amniotic fluid (7).

A surgical and invasive procedure must be used to collect stem cells from bone marrow and adipocytes. However, placenta, umbilical cord and AM, which are generally considered medical waste, can be obtained without the need for an invasive procedure and can be used as a stem cell source (8). In addition, they do not raise ethical concerns as they are usually discarded after delivery. Therefore, amnion-derived cells are expected to be a valuable cell source for regenerative medicine studies. Since amniotic membrane is a waste material and mesenchymal stem cells can be easily derived with this technique, it may be the most widely used stem cell source candidate for personalized therapies (derived exosomes from mesenchymal stem cells) in the future (9). Epithelial and mesenchymal amnion cells have pluripotent potential (7).

Amniotic epithelial cells have the capability to differentiate into neurons, glial cells and alveolar epithelial cells. Some studies have reported hepatic, cardiac, osteogenic, chondrogenic and adipogenic differentiation of amniotic epithelial cells. Since MSCs in the AM are located at the interface between the maternal and fetal organisms, they have a high immunologic tolerance, facilitating their use in transplantation.

One of the mechanisms providing immunologic tolerance of the maternal organism against the fetal organism is the reduction of the expression of HLA molecules on the surface of fetal cells and some placental cells (10). AM can be used in ocular surface reconstruction and wound healing in the clinic in terms of promoting reepithelialization, inhibiting angiogenesis and reducing inflammation (6). Studies have shown that human amniotic membrane-derived stem cells (AM-SC) support sciatic nerve repair (a classic peripheral nerve regeneration model) (11).

Mesenchymal Stem Cell Exosomes Derived from Amniotic Membrane

Exosomes are 40 to 100 nm membrane vesicles that paracrinally deliver bioactive components such as RNA and proteins from stem cells to surrounding cells. These vesicles mediate the exchange of functional proteins, lipids, mRNAs and microRNAs (miRNAs) between cells (11,12). Culture supernatants of stem cells contain exosomes (12). It has been shown that MSC exosomes can act as a therapeutic secretion that helps reduce tissue damage (13).

Studies have shown that human amniotic membrane mesenchymal stem cells (AM-MSCs) support fibroblast migration. After exosome inhibitor was added to the culture, fibroblast migration was reduced and AM-MSCs exosomes were found to be involved in fibroblast migration (12).

Mesenchymal Stem Cell Isolation Methods

The procedure developed for MSC isolation is an important step for clinical applications. Different protocols are used to isolate MSCs, depending on the cell source and the use of the isolated cells (14). Isolation methods are divided into 2

main groups: enzymatic method and explant culture method. In the enzymatic method, proteolytic enzymes are used to separate cells from tissue and cultured in appropriate medium. In the explant method, no enzymes are used. The tissue is cut into small pieces in a culture dish. Reducing the size of the tissue pieces facilitates the diffusion of gases and nutrients towards the cells.

Studies have shown that the explant method has a higher rate of proliferation, increased cell viability and purer stem cells than the enzymatic method (15-17). This may be due to the presence of extracellular matrix in the tissue pieces in explant culture. As a result, cells are protected from proteolytic and mechanical stress and a favorable environment is provided for migrating cells. In addition, cytokines and growth factors continue to be released into the medium (16).

The aim of our study was to non-invasively isolate MSCs from the postnatal AM by explant culture method without using any enzyme. Thus, these cells may be an alternative to the MSCs isolated from the umbilical cord, which are now widely used in regenerative and reparative medicine.

MATERIALS and METHODS

Our study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee with the decision numbered E.201304 dated 28.04.2022. Two healthy female patients between the ages of 25-30 years, who gave birth and had no systemic disease were included in our study after written informed consent was obtained.

Dublecco's Modified Eagle Medium ((DMEM), Thermofisher, Cat no:11965092, USA) penicillin-streptomycin (Capricorn Scientific, Cat no: PS-B, Germany) and fetal bovine serum ((FBS), Thermofisher, Cat no: A5256701, USA) were placed in a falcon tube. The amniotic membrane was brought to the cell culture laboratory within 10 minutes after removal from the delivery room. The amniotic membrane was placed in a falcon tube prepared under sterile conditions in a laminar flow cabinet and was washed with Phosphate Buffered Saline (PBS, Capricorn Scientific, Cat no: PBS-2A, Germany) to completely remove cell debris and blood. The amniotic membrane was dissected into small pieces with a sterile scalpel and forceps, placed in a petri dish containing complete medium and monitored in a humidified incubator with 5% CO_2 at 37°C. The medium in the petri dish was carefully changed once every two days. The complete medium contained DMEM, 10% FBS, 50U/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine and 0.1 mM nonessential aminoacids.

The migration of cells around the amniotic membrane tissue fragments and their adhesion and proliferation were monitored. Cells started to migrate on day 3 and became confluent on day 10. The tissue pieces in the petri dish were removed and the confluent cells were trypsinized ((1 Mm EDTA containing 0.25% trypsin) Wisent Inc. Cat no: 325-542 EL, USA) and removed from the bottom of the petri dish. These cells were then added equally to other Petri dishes and proliferated by repeated passages. Flow cytometry analysis was performed at the 3rd passage (P3) to determine the characterization of the proliferating cells.

Cells were sent to Pamukkale University Hospital Tissue Typing Laboratory for mesenchymal stem cell characterization. Cells were characterized by Flow Cytometry (Navios EX, Beckman Coulter Life Sciences, Houston, Texas) using CD34, CD73 and CD90 hematopoietic and mesenchymal stem cell surface markers. For the validation of these analysis results mesenchymal stem cell differentiation experiments were performed. All these steps were observed using invert microscope (CKX41, Olympus, Japan). The proliferating cells were counted in Thoma cell counting chamber after staining with trypan blue.

Differentiation experiments

Differentiation assays were performed at passage 3 to demonstrate changes in adipogenic, chondrogenic and osteogenic direction. StemPro Adipogenesis Differentiation Kit, StemPro Osteogenesis Differentiation Kit and StemPro Chondrogenesis Differentiation Kit (all ready-to-use kits obtained from Gibco, Invitrogen Cell Culture, Carlsbad, USA) were used according to the appropriate protocols to demonstrate the adipogenesis, osteogenesis and chondrogenesis potential of amnion-derived MSCs, respectively. Adipogenesis was demonstrated by Oil Red O staining, osteogenesis by Alizarin Red S staining and chondrogenesis by Alcian Blue staining (all from Sigma-Aldrich Chemie GmbH, Germany).

Adipogenic differentiation and oil red O staining

Third-passage culture-expanded cells at 70% confluence were induced in the following adipogenic mediums for 15 days. The adipogenic medium (45 ml basal medium, 5 ml adipocyte supplement, 25 μl penicillin–streptomycin) was prepared and changed every 3 days. Adipogeneses were determined by post induction oil red O staining. Cells were washed twice with PBS and fixed with 10% formalin for 10 min. After fixation, cells were stained with aliquots of filtered oil red O solution (stock solution: 3 mg/ml in isopropanol; working solution: 60% oil red O stock solution and 40% distilled water) for 1 h. After staining, cells were washed with water to remove unbound dyes. Hematoxylin–Eosin was added to each box for 1 min and then washed with distilled water. Cells visualized by phase contrast microscope and photographed.

Osteogenic differentiation and Alizerin Red S staining

After removing the cells in the third passage and counting the cells, there were 3.5×10^6 viable cells in 1 ml of cell suspension. After inserting medium (1,813,285; Gibco, USA) into a culture dish and adding 20 μl of cell suspension, the culture dish was placed in the incubator. The medium was changed every 2–3 days. At the end of the 21st day, after removing the medium, 4% formaldehyde was added to the cells and left for 30 min. After fixation, stained with Alizerin Red S (E1816; ChemCruz, SantaCruz, USA) for 2–3 min. Cells visualized by phase contrast microscope and photographed.

Chondrogenic differentiation and alcian blue staining

Cells in the third passage were removed and counted. Five droplets which had five microliters of the cell suspension prepared at a concentration of 1.6×10^7 live cells/ml were placed in the center of 12 well-plate wells and incubated at 37 °C for 2 h. Then, the chondrogenic differentiation medium (1,813,309; Gibco, USA) added on it and placed in the incubator. The medium was changed every 2–3 days (twice a week). At the end of the 14th day, after removing the medium, 4% formaldehyde was added to the cells and left for 30 min. After fixation, the cells were incubated in 1% Alcian blue (SLBR0633V; SigmaAldrich, India) solution dissolved in 0.1 N HCl for 30 min. Alcian Blue was removed and cells washed with 0.1 N HCl then cells were visualized by Phase contrast microscope (18).

All these steps were observed using an inverted microscope (CKX41 Olympus, Japan). Pictures were taken with a light microscope (Olympus BX51 light microscope and Olympus DP72 camera). Cell numbers were determined with a Neubauer Improved counting chamber.

RESULTS

In our study, explant culture technique was used for the isolation of AM-MSCs. Amniotic tissue was dissected into small pieces and seeded on plates and no enzyme was used (Figure 1). Cells started to migrate on day 3. The cells become confluent on day 10. When examined by phase contrast

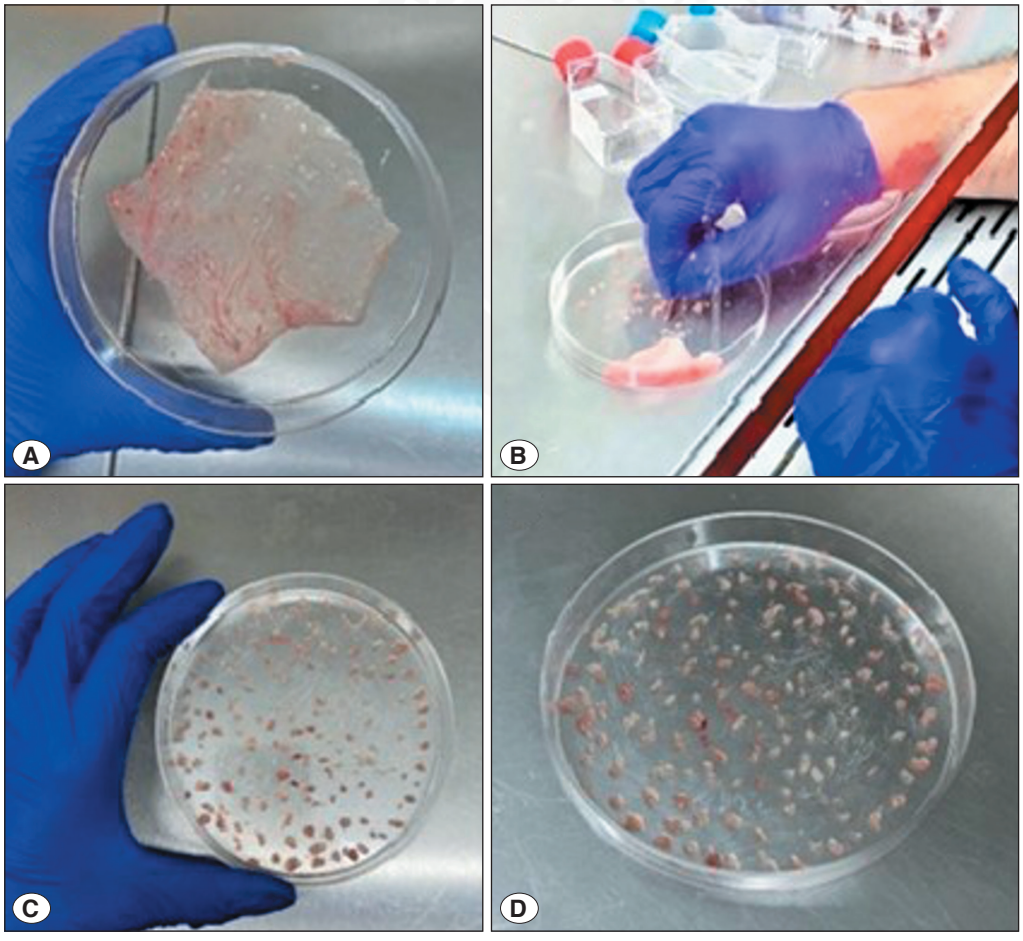

Figure 1: Isolation of mesenchymal stem cells from amniotic membrane by explant method. Amniotic membrane taken under sterile conditions is placed in a petri dish in a sterile environment **(A).** It is divided into small pieces with a scalpel **(B).** The pieces adhere to the bottom of the petri dish over time and mesenchymal stem cells migrate and start to proliferate around these pieces **(C,D).**

microscopy, they were observed as spindle-shaped and fibroblast-like cell aggregates. (Figure 2). Approximately 1.4x 10^6 cells were counted on one plate and 1.2x 10^6 cells on the other plate. The cells in the Petri dish were removed from the bottom of the dish with trypsin and passaged (Figure 3). Flow cytometry analysis was performed at the 3rd passage (P3) to determine the characterization of proliferating cells.

Differentiation experiments were performed to show the changes in adipogenic, chondrogenic and osteogenic direction. Differentiation kits were applied according to appropriate protocols.

As a result, it was determined that the cells showed adipogenic, chondogenic and osteogenic changes (Figure 4).

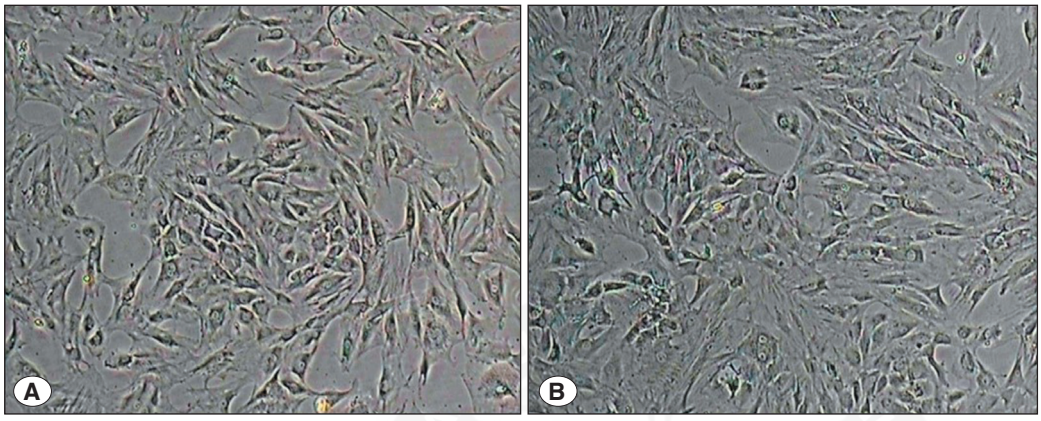

Figure 2: The proliferation of amniotic membrane mesenchymal stem cells isolated by explant culture **(A,B)** (Day 10, x200)

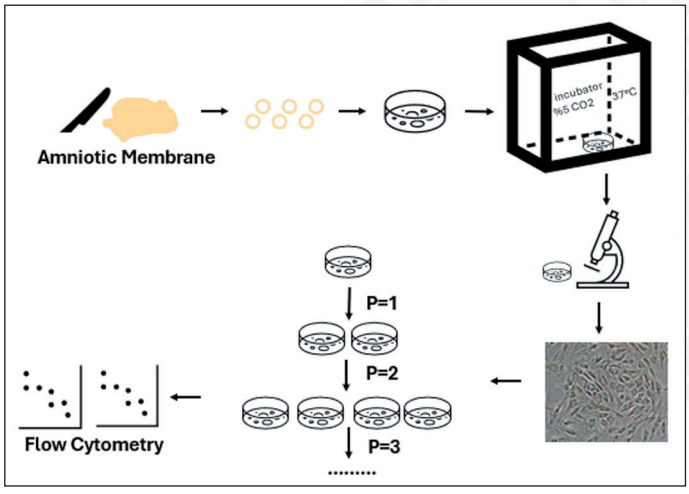

Figure 3: Schematic image of isolation of mesenchymal stem cells from human amniotic membrane by explant cell culture technique. In sterile media, amniotic tissue is dissected into small pieces and seeded on plates and monitored in a 37°C humidified incubator with 5% CO2. After the cells become confluent, they are lifted from the bottom of the plate with trypsin and passaged. Flow cytometry analysis is performed at passage 3 (P3) to determine the characterization of proliferating cells.

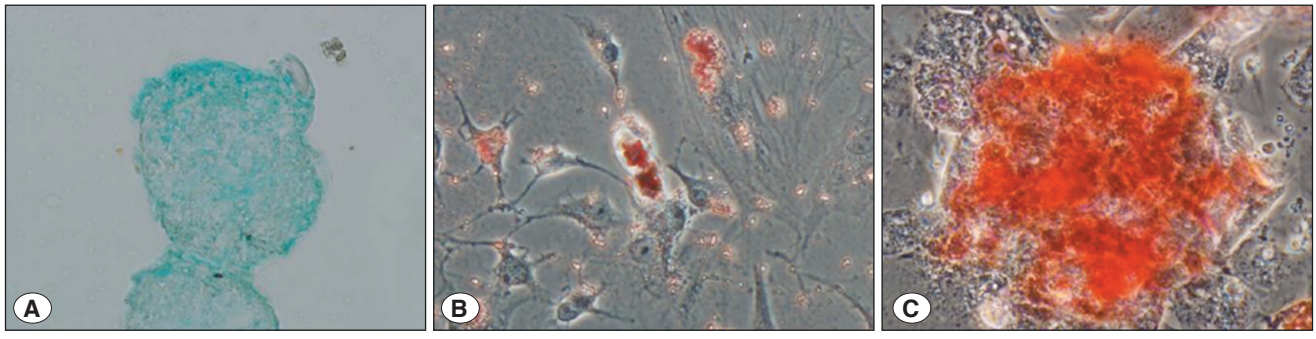

Figure 4: Cells maintained in a chondrogenesis differentiation medium for 14 days were positively stained with Alcian Blue (x400) **(A).** Cells maintained in adipogenesis differentiation medium for 15 days contained lipid droplets positively stained with Oil Red O (x400) **(B).** Cells maintained in osteogenesis differentiation medium for 21 days were positively stained with Alizarin Red S. (x400) **(C).** As a result, it was determined that the cells showed adipogenic, chondogenic and osteogenic changes.

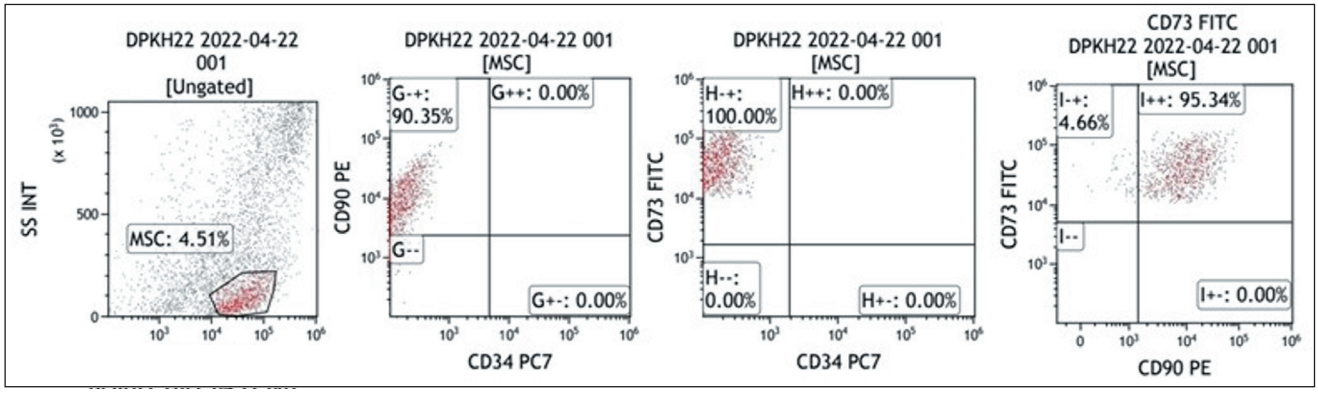

Figure 5: Expression of surface markers of human amniotic membrane-derived MSCs measured by flow cytometry analysis. CD73 was 95.34% and CD90 was 90.35%. CD34 expression was not observed.

Flow cytometry analysis showed that the cells expressed MSC markers (CD73 and CD90) but not hematopoietic stem cell markers (CD34) (Figure 5).

DISCUSSION

In the enzymatic method, which is one of the stem cell isolation methods, proteolytic enzymes such as trypsin, dispases and/or collagenases are used to separate the cells from the tissue and culture in appropriate medium (15,19,20). In the explant method, the tissue is divided into small pieces in a culture dish. It is cultured in an appropriate medium. Afterwards, cells migrate from the tissue and adhere to the culture surface (15,17). Yoon et al. showed that explant culture was superior to the enzymatic method in terms of yield and viability of MSCs (19).

In the study by Lee et al. distal femur synovial tissue samples of patients with osteoarthritis were collected and the collected tissue was divided into 2 equal parts. Half of the tissue was used for MSC isolation by enzymatic method and the other half by explant method. Both enzymatic and explant techniques produced MSCs with similar properties and yield. However, they thought that the explant method was preferable to the enzymatic method for isolating MSCs because it was simpler and non-invasive. In addition, since only migratory MSCs were present in synovial explant culture, contamination by other cells was found to be minimal compared to enzymatically produced MSCs (20).

Collagenase purified from bacteria for use in the enzymatic method may be contaminated with endotoxin in bacteria. This may inhibit the proliferation of MSCs (20,21). In our study, MSCs were isolated from AM by explant culture technique quickly, easily, without the need for much equipment and reliably.

In the study by Ma et al. MSCs were isolated from the AM, umbilical cord and chorionic plate from the same donor and their differentiation capacities were compared. These three neonatal MSCs were shown to have similar morphology and immunophenotype. However, AM-MSCs were shown to have a higher capacity for osteogenic differentiation. Chorionic membrane-derived MSCs showed a higher capacity for better adipogenic differentiation. All of these three neonatal MSCs showed similar chondrogenic potential (22). Seo et al. demonstrated that MSCs generated from the equine AM by enzymatic method exhibited a phenotype similar to human MSCs and self-renewal (23).

Koike et al. compared human amnion-derived epithelial cells and human amnion-derived mesenchymal cells with bone marrow-derived mesenchymal stem cells (BM-MSCs). It was concluded that amnion-derived stem cells are more similar to pluripotent stem cells than BM-MSCs. In this study, AM-SCs were isolated from the AM at 35 to 36 weeks of gestation. If these cells are isolated from the AM at an earlier stage, they may express more stem cell markers. However, the use of amnion at an earlier stage may raise ethical issues as it is a consequence of abortion (9).

Ding et al. showed that hAM-MSCs increased the number of follicles in the mouse ovary and showed a therapeutic activity on ovarian function (24). In the study by Seong et al. human were derived by hypoxic culture (2% O_2) in a conditioned medium containing more exosome particles. They found that the derived cells protected retinal cells against oxidative and hypoxic damages in vitro and improved glaucoma by restoring intraocular pressure and retinal degeneration in vivo (25).

In the study by Noh et al., human AM-SC were cultured under low oxygen (2% O_2) and collected in exosome-rich conditioned medium (ERCM). In the cell migration experiment, a scratch on a keratinocyte culture dish was treated with ERCM and rapidly closed. It was observed that ERCM accelerated wound closure in rats. In the wounded skin, collagen accumulation was supported and fibroblasts were activated (26). Kim et al. aimed to evaluate the efficacy of (MSCs) and

MSC-derived exosomes (MSC-Exos) to promote the healing of large and treatment-resistant macular holes (MH). They showed that intravitreal injection of MSCs and MSC-Exos may improve the outcomes of surgery for refractory MHs (27).

In conclusion, the biggest advantage of AM-MSCs compared to other tissue-derived MSCs in cultures is the higher self-renewal capacity and the ability to access a large number of cells in a shorter time. These cells, which also express pluripotency markers of embryonic stem cells at early passages, are between adult stem cells and embryonic stem cells. The AM is a material used as medical waste after birth. Since these MSCs are easily derived by a non-invasive method, they may find wide use in regenerative and reparative medicine in the future.

The findings of this study support the potential clinical utility of mesenchymal stem cells derived from human amniotic membrane. Especially in the fields of immunomodulation, tissue regeneration, and cellular therapy, further investigations are warranted to evaluate their safety and efficacy. Future studies may focus on therapeutic applications in disease models, standardization of GMP-based production processes, and immunogenic profile assessments. These efforts will help clarify the clinical relevance of amnion-derived stem cells. Moreover, quantitative comparison of mesenchymal stem cell surface marker expression among cells derived from fetal tissues such as the umbilical cord, amniotic membrane, and placenta may provide valuable insights into their phenotypic similarities and differences. Such expression profiling can generate objective and comparable data to evaluate the suitability of each cell source for tissue engineering and cell-based therapies. Performing these analyses under standardized conditions may help identify the most clinically advantageous MSC source for regenerative applications.

Although this study characterized mesenchymal stem cells (MSCs) derived from the amniotic membrane, it did not include an analysis of pluripotency gene expression. Nevertheless, previous studies have reported low-level expression of key pluripotency-associated transcription factors such as *OCT4*, *SOX2*, *NANOG*, and *KLF4* in MSCs, particularly those derived from fetal sources. This suggests a need for comparative investigations among different fetal-derived MSCs—including amniotic membrane, umbilical cord, and placenta—to better understand their respective regenerative capacities. Future studies assessing pluripotency gene expression profiles and differentiation potential could provide valuable insights for selecting optimal MSC sources for regenerative and reconstructive medicine.

There are few studies on mesenchymal stem cell isolation from amniotic membrane by explant method. Therefore, we established our own method in our study and this constituted the limitation of our study.

Acknowledgments

None.

Author Contributions

Concept: **Murat Serkant Ünal, Hale Yetgin,** Design: **Murat Serkant Ünal, Hale Yetgin, Elif Önder, Cihan Kabukçu, Ergun Mete,** Data collection or processing: **Murat Serkant Ünal, Hale Yetgin, Elif Önder, Cihan Kabukçu, Ergun Mete,** Analysis or Interpretation: **Murat Serkant Ünal, Hale Yetgin, Elif Önder, Cihan Kabukçu, Ergun Mete,** Literature search: **Murat Serkant Ünal, Hale Yetgin, Elif Önder, Cihan Kabukçu, Ergun Mete,** Writing: **Murat Serkant Ünal, Hale Yetgin, Elif Önder, Cihan Kabukçu, Ergun Mete,** Approval: **Murat Serkant Ünal, Hale Yetgin, Elif Önder, Cihan Kabukçu, Ergun Mete.**

Conflicts of Interest

No conflict of interest was declared by the authors.

Financial Support

The authors declared that this study has received no financial support.

Ethical Approval

Our study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee with the decision numbered E.201304 dated 28.04.2022.

Review Process

Extremely and externally peer-reviewed.

REFERENCES

1. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ. Safety of Cell Therapy With Mesenchymal Stromal Cells (Safecell): A Systematic Review and Meta-Analysis of Clinical Trials. PLoS One. 2012;7:47559. https://doi:10.1371/journal.pone.0047559.
2. Rastegar F, Shenaq D, Huang J, Zhang W, Zhang BQ, He BC, Chen L, Zuo GW, Luo Q, Shi Q, Wagner ER, Huang E, Gao Y, Gao JL, Kim SH, Zhou JZ, Bi Y, Su Y, Zhu G, Luo J, Luo X, Qin J, Reid RR, Luu HH, Haydon RC, Deng ZL, He TC. Mesenchymal Stem Cells: Molecular Characteristics and Clinical Applications. World J Stem Cells. 2010;26:67-80. https://doi:10.4252/wjsc.v2.i4.67.
3. Ding DC, Chang YH, Shyu WC, Lin SZ. Human Umbilical Cord Mesenchymal Stem Cells: a New Era for Stem Cell Therapy. Cell Transplant. 2015;24:339-47. https://doi:10.3727/096368915X686841.
4. Kabasakal G, Tural E, Unal MS. The Effect of Mesenchymal Stem Cells on Ovarian Tissue in Experimental Ovarian Failures. Kocatepe Medical Journal. 2023;24,249-53. https://doi.org/10.18229/kocatepetip.849512.
5. Roubelakis MG, Tsaknakis G, Pappa KI, Anagnou NP, Watt SM. Spindle Shaped Human Mesenchymal Stem/Stromal Cells from Amniotic Fluid Promote Neovascularization. PLoS One. 2013;8:54747. https://doi: 10.1371/journal.pone.0054747.

6. Maymo JL, Riedel R, Perez-Perez A, Magatti M, Maskin B, Duenas JL, Parolini O, Sánchez-Margalet V, Varone CL. Proliferation and Survival of Human Amniotic Epithelial Cells During Their Hepatic Differentiation. PLoS One. 2018;13:0191489. https://doi: 10.1371/journal.pone.0191489.
7. Mamede AC, Carvalho MJ, Abrantes AM, Laranjo M, Maia CJ, Botelho MF. Amniotic Membrane: from Structure and Functions to Clinical Applications. Cell Tissue Res. 2012; 349:447-58. https://doi: 10.1007/s00441-012-1424-6.
8. Seo MS, Park SB, Kim HS, Kang JG, Chae JS, Kang KS. Isolation and Characterization of Equine Amniotic Membrane-Derived Mesenchymal Stem Cells. J Vet Sci. 2013;14:151-9. https://doi: 10.4142/jvs.2013.14.2.151.
9. Koike C, Zhou K, Takeda Y, Fathy M, Okabe M, Yoshida T, Nakamura Y, Kato Y, Nikaido T. Characterization of Amniotic Stem Cells. Cell Reprogram. 2014;16:298-305. https://doi: 10.1089/cell.2013.0090.
10. Mihu CM, Rus Ciuca D, Soritau O, Susman S, Mihu D. Isolation and Characterization of Mesenchymal Stem Cells from the Amniotic Membrane. Rom J Morphol Embryol. 2009;50:73-7.
11. Chen W, Chang S, Yang C, Zhou J, Zhang H, Nie K, Wei Z. Schwann CellLike Cells Derived from Human Amniotic Mesenchymal Stem Cells Promote Sciatic Nerve Repair Through an Exosomeİnduced SOX2/FN1 Pathway İn Vitro. Int J Mol Med. 2022;49:80. https://doi.org/10.3892/ijmm.2022.5136.
12. Chen T, Gao S, Hao Y, Zhang F, Tang X, Wei Z, Wang D, Qi J. Experimental Study of Human Amniotic Mesenchymal Stem Cell Exosome Promoting Fibroblasts Migration Through Microrna-135a. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2020;34:234-9. https://doi.org/10.7507/1002-1892.201907136.
13. Doyle LM, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells. 2019;8:727. https://doi: 10.3390/cells8070727.
14. Chan TM, Harn HJ, Lin HP, Chou PW, Chen JY, Ho TJ, Chiou TW, Chuang HM, Chiu SC, Chen YC, Yen SY, Huang MH, Liang BC, Lin SZ. Improved Human Mesenchymal Stem Cell İsolation. Cell Transplant. 2014;23:399-406. https://doi.org/10.3727/096368914X678292.
15. Hendijani F. Explant culture: An Advantageous Method for İsolation of Mesenchymal Stem Cells from Human Tissues. Cell Prolif. 2017;50:12334. https://doi.org/10.1111/cpr.12334.
16. Mushahary D, Spittler A, Kasper C, Weber V, Charwat V. Isolation, Cultivation and Characterization of Human Mesenchymal Stem Cells. Cytometry A. 2018;93:19-31. https://doi.org/10.1002/cyto.a.23242.
17. Pirjali T, Azarpira N, Ayatollahi M, Aghdaie MH, Geramizadeh B, Talai T. Isolation and Characterization of Human Mesenchymal Stem Cells Derived from Human Umbilical Cord Wharton's Jelly and Amniotic Membrane. Int J Organ Transplant Med. 2013;4:111-6.
18. Çil N, Yaka M, Ünal MS, Dodurga Y, Tan S, Seçme M, Karagür ER, Mete GA. Adipose Derived Mesenchymal Stem Cell Treatment in Experimental Asherman Syndrome İnduced Rats. Mol Biol Rep. 2020;47:4541-52. https://doi:10.1007/s11033-020-05505-4.
19. Yoon JH, Roh EY, Shin S, Jung NH, Song EY, Chang JY, Kim BJ, Jeon HW. Comparison of Explant-Derived and Enzymatic Digestion-Derived Mscs and the Growth Factors from Wharton's Jelly. Biomed Res Int. 2013;1:428726. https://doi:10.1155/2013/428726.
20. Lee DH, Joo SD, Han SB, Im J, Lee SH, Sonn CH, Lee KM. Isolation and Expansion of Synovial CD34(-)CD44(+)CD90(+) Mesenchymal Stem Cells: Comparison of an Enzymatic Method and a Direct Explant Technique. Connect Tissue Res. 2011;52:226-34. https://doi:10.3109/03008207.2010.516850.
21. Park SG, Kim JH, Oh JH, Lee HN, Park HS, Chung SS, Lee YJ, Lee YY, Jung HS, Park KS. Polymyxin B, Scavenger of Endotoxin, Enhances İsolation Yield and İn Vivo Function of İslets. Transpl Int. 2010;23:325-32. https://doi:10.1111/j.1432-2277.2009.00987.x.
22. Ma J, Wu J, Han L, Jiang X, Yan L, Hao J, Wang H. Comparative Analysis of Mesenchymal Stem Cells Derived From Amniotic Membrane, Umbilical Cord and Chorionic Plate Under Serum-Free Condition. Stem Cell Res Ther. 2019;10:19. https://doi:10.1186/s13287-018-1104-x.
23. Seo MS, Park SB, Kim HS, Kang JG, Chae JS, Kang KS. Isolation and Characterization of Equine Amniotic Membrane-Derived Mesenchymal Stem Cells. J Vet Sci. 2013;14:151-9. https://doi: 10.4142/jvs.2013.14.2.151.
24. Ding C, Zou Q, Wang F, Wu H, Chen R, Lv J, Ling M, Sun J, Wang W, Li H, Huang B. Human Amniotic Mesenchymal Stem Cells İmprove Ovarian Function in Natural Aging Through Secreting Hepatocyte Growth Factor and Epidermal Growth Factor. Stem Cell Res Ther. 2018;9:55. https://doi:10.1186/s13287-018-0781-9.
25. Seong HR, Noh CH, Park S, Cho S, Hong SJ, Lee AY, Geum D, Hong SC, Park D, Kim TM, Choi EK, Kim YB. Intraocular Pressure-Lowering and Retina-Protective Effects of Exosome-Rich Conditioned Media from Human Amniotic Membrane Stem Cells in a Rat Model of Glaucoma. Int J Mol Sci. 2023;24:8073. https://doi:10.3390/ijms24098073.
26. Noh CH, Park S, Seong HR, Lee AY, Tsolmon KE, Geum D, Hong SC, Kim TM, Choi EK, Kim YB. An Exosome-Rich Conditioned Medium from Human Amniotic Membrane Stem Cells Facilitates Wound Healing via Increased Reepithelization, Collagen Synthesis, and Angiogenesis. Cells. 2023;12:2698. https://doi:10.3390/cells12232698.
27. Kim H, Goh YS, Park SE, Hwang J, Kang N, Jung JS, Kim YB, Choi EK, Park KM. Preventive Effects of Exosome-Rich Conditioned Medium From Amniotic Membrane-Derived Mesenchymal Stem Cells for Diabetic Retinopathy in Rats. Transl Vis Sci Technol. 2023;12:18. https://doi:10.1167/tvst.12.8.18.