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Comparison characteristics of the epithelial and mesenchymal stem cells derived from the amniotic membrane of human

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ABSTRACT

The amniotic membrane has been confirmed as a source of the epithelial and mesenchymal stem cells, which display many characteristics of the embryonic stem cells (ESCs) and this promoted researchers to study their characteristics in detail, hoping to be an alternative source of the ESCs. The amniotic membrane stem cells have different appearance and embryonic origin. Properties of the amniotic membrane mesenchymal stem cells (MSCs) have been studied intensively, without comparison of their characteristics with epithelial stem cells. Therefore, the present literature review is concerned with comparing their neurological properties and role in angiogenesis. This comparison may provide an approach to study the biology of each type of the amniotic membrane stem cell precisely, which in turn facilitates their employment in the clinical applications.

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1. INTRODCTION

Recently, most investigations confirmed the benefits of stem cells in regenerative medicine, due to their unique properties such as unlimited self- renewal and differentiation potential into multiple cell lineages or more restricted progenitor's population that can contribute to tissue homeostasis by replenishment of cells or regeneration of tissue after injury [1]. For instance treatment of many battlefield injuries and their complications, including treatment of injuries to the skin, nervous system tissues, sensory organs, the musculoskeletal system, circulatory/pulmonary tissues and genitals/ testicles in addition to the acute radiation syndrome and the development of novel biosensors [2].

Based on the origin, stem cells can be classified into two types, ESCs and adult stem cells (ASCs) [3]. It is well known that ESCs are pluripotent cells and able to differentiate into three types of germ layer cells however, harvesting these cells requires the destruction of human embryos, a practice associated with substantial moral and ethical issues [4, 5]. Moreover, some studies described the tumorigenic of undifferentiated human embryonic stem cells (hESCs) and the possibility of teratoma formation [6]. These issues evoked the scientists to search for alternative sources of ESCs, and other sources of ESCs were ASCs, these cells are derived from different tissues in fetuses, children and adults [7, 4, 8]. Umbilical cord blood and bone marrow represent important sources for ASCs [9], and gradually became the tissues of increasing interest due to their characteristics that make them potential candidates for use in regenerative medicine .It has been reported that two types of stem cells reside in umbilical cord blood and bone marrow, hematopoietic and mesenchymal stem cells (HSCs and MSCs) [9]. In addition to these sources, MSCs are found in all organs having connective tissue components and in almost all types of mesenchymal tissues [10]. Therefore these cells have been harvested from adipose tissue [11], human dental pulp and periodontal ligament [12]. MSCs are

easily extracted from bone marrow, adipose tissue, and synovium, and differentiate into various cell lineages according to the requirements of specific biomedical applications [13]. There are many characteristics that make MSCs safe and promising candidates for use in regenerative medicine such as a broad range of distribution, these cells are free from ethical issues, possess non- immunogenic properties, tend to migrate and engraft at injury sites, and can be used as vehicles for gene therapy [14].

For these reasons, literature dealing with MSCs and their applications has increased dramatically, indeed a considerable number of these cells are indispensable for treatment of diseases and according to previous studies, harvesting of MSCs from human bone marrow is a surgical procedure that requires general anesthesia or sedation, yields a limited number of cells as the percentage of MSCs in the bone marrow is very low (0.001-0.01%). Furthermore, the number of these cells and their proliferation and differentiation potentials decrease with age [8, 15, 16, 17]. Hence, perinatal stem cells have generated more professional interest than any other sources of stem cells, they refer to stem cells derived from umbilical cord blood, Warton's jelly, and chorion, amniotic fluid and amniotic membrane [18, 19].

Currently, stem cells isolated from amniotic membrane (AM) have obtained growing interest because they are isolated via noninvasive methods, free from ethical issues and assumed to exhibit multilineage differentiation potential. Stem cells of the amnion membrane can be classified into two sorts: amniotic membrane epithelial stem cells (AMESCs) and mesenchymal stromal stem cells (AMSSCs) [15, 16, 20, 21]. These cells have characteristics of pluripotent stem cells such as their ability to differentiate into all three germ layers and can be obtained without any ethical concern [22]. The previous studies explained the characteristics of each type of stem cell derived from AM in detail [23, 20, 21], such as morphological and immunophenotype changes that occur in cultivated epithelial cells culture isolated from AM, genetic analysis that described subpopulation of genes in these cells characteristic to hepatocytes, and their differentiation into myocytes, cardiomyoctes ,neural cells and insulin producing cells [24, 16]. The AM and its epithelial and mesenchymal stem cells, as demonstrated by [22] being one of the best candidates to be employed in skin tissue engineering and regenerative medicine. There was a new trend to compare characteristics, differentiation potential and clinical applications of AMSCs with MSCs derived from umbilical cord blood, all parts of placenta, bone marrow and adipose tissue [25, 19, 26]. MSCs isolated from different sources have different biological properties and genetic backgrounds, while there is a lack of genetic differences among perinatalMSCs [27].

Since both types of stem cells are derived from the AM and considered as a valuable source of stem cells for a broad range of clinical applications, therefore this review focused on the angiogenic potential and neural characteristics of these cells that might be important due to their therapeutic effects

2. AMNIOTIC MEMBRANE STRUCTURE AND PROPERTIes

The amniontic membrane is composed of five layers and these are a single epithelial layer, thick basement membrane, compact layer, fibroblast layer and spongy layer (Figure 1) [28, 29, 30, and 21]. After implantation of the human blastocyst in the endometrial stroma, the inner cell mass differentiates into two layers, the hypoblast and epiblast, and both the developing embryo and amniotic epithelial cells derived from epiblast [31]. At the same time, the embryonic mesoderm gives rise to AMSCs (Figure 2) [32].

The single epithelial layer of the AM includes a homogenous population of cells [33]. In contrast, the underlying basement membrane of the epithelial cells is composed of collagen types III, IV, V, laminin and fibronectin [30]. The immunochemical analysis has demonstrated that the epithelium is active in the production of a mixture of basal laminal and stromal components [34]. Consequently, the structure and the unique properties of the amnion have been investigated, particularly the amnion can be a promising candidate to provide scaffolds necessary for tissue engineering [30, 35, 36]. The amnion possess properties such as anti-microbial, anti- inflammatory effects and pro-apoptotic ability [37]. Beside these properties, the amnion enhances epithelialization and exhibits anti-scar, therefore it is used for treatment of burns of face, neck and elsewhere [38], to accelerate wound healing [39], and to improve the viability of flap in rat model of random skin flap by using the amnion matrix scaffolds with bone marrow derived mesenchymal stem cells (BM-MSC) [36].

2.1 Immunophenotype characterization of amniontic membrane derived stem cells

Most studies deal with cultures of amniotic epithelial cells are used cytokeratins as an informative marker to confirm their epithelial nature [40, 41, 42, 43]. A detailed immunophenotype analysis of stem cells surface markers present on cultured hAEcs were performed and the results demonstrated that hAEcs express

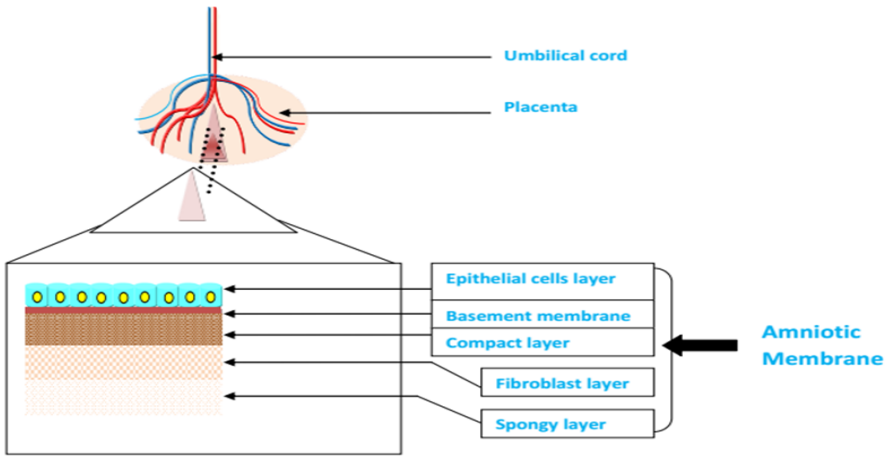

Figure1. Schematic diagram shows the elements that constitute the amniotic membrane

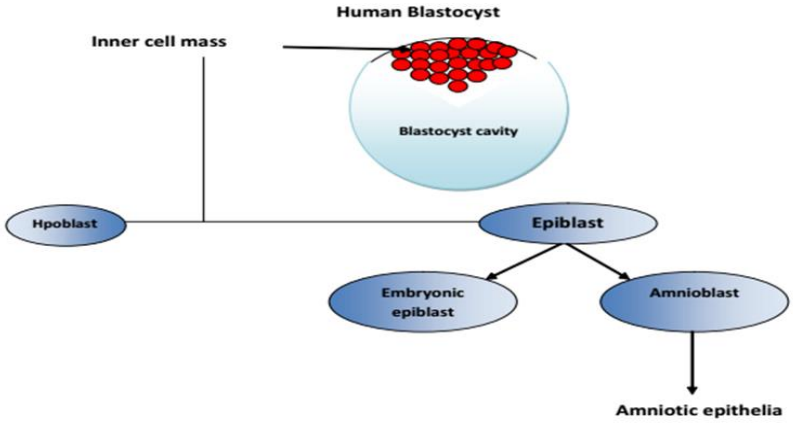

Figure 2. Schematic diagram elucidates how amniotic epithelia derive from epiblast during eraly stages of embryonic development

SSA-3, SSA-4, TRA1-60 and TRA-81[44, 41, 43, 45], Sox-2[46] together with the expression of stem cells pluripotent –specific transcriptions octamer-binding protein-4(Oct-4) and Nanog[44, 41, 42, 43, 46, 45] . Moreover, hAECs express CD44, CD90, CD73, CD10, CD13,CD 29, CD49, CD117, CD105 and CD 166 [16,47],however numerous publications revealed that hAECs did not express CD34 [42],CD14, CD45 and HLA-DR [15].

Based on several reports,cultured epithelial cells through serial passages acquired mesenchymal –stromal morphology and this phenomennon was confirmed by down regulation of epithelial markers such as CD49f and E-Caderin and elevation of some mesenchymal markers such as Vimentin, CD105and CD 44[48]. The immunophenotypic analysis of mesenchymal- stromal cells derived from human amnion membrane has demonstrated that these cells express SSEA-4, OCT-4, SOX-2, CD105, C29, CD73, CD49d , CD 166 and Vimentin[23,6] Moreover, approximately 50% of cultured hAMSC exhibited epithelial marker cytokeratin -7 beside mesenchymal marker vimentin marker[49].This would be consistent with a previous report that displayed mesenchymal stem cells derived from human term placenta possess both epithelial and mesenchymal ultrastructural phenotype[50].These results have been evaluated precisely and some reports suggested the impact of trypsin which is used to release the adhesive cells to stimulate proteome changes for instance, about 36 proteins and some CD markers are expressed at different levels in the cells after treatment by trypsin , some of these proteins are situated in the plasma membrane [41,51]. There was a lack of consensus among investigators about the phenotype of MSCs due to their isolation from various tissues and their cultivation under different culture conditions, which may affect the phenotype of MSCs[52].Therefore, the mesenchymal and tissue stem cell committee of the international society for cellular therapy suggested that the fibroblast-like plastic adherent cells is termed multipotent MSCs regardless of the tissue from which they are derived

[53]in order to provide a sufficient approach for studying MSCs biology the international society for cellular therapy proposed limited criteria to distinguish human MSCs (hMSCs) such as plastic adherent, expression of CD105,CD73and CD90, must lack expression of CD45, Cd34, CD14, CD11b, CD79, CD19 and HLA-DR surface molecules as well as multi-lineage differentiation potential to osteoblasts, adipocytes and chondroblasts [54]. Hence, the recommendations of the first international workshop on placenta derived stem cells were in line with the limited criteria that proposed for identification of mesenchymal stem cells [15].

Based on the immunophenotype analysis mentioned above, identification markers of amnion membrane derives stem cells can be summarized in Figure 3.

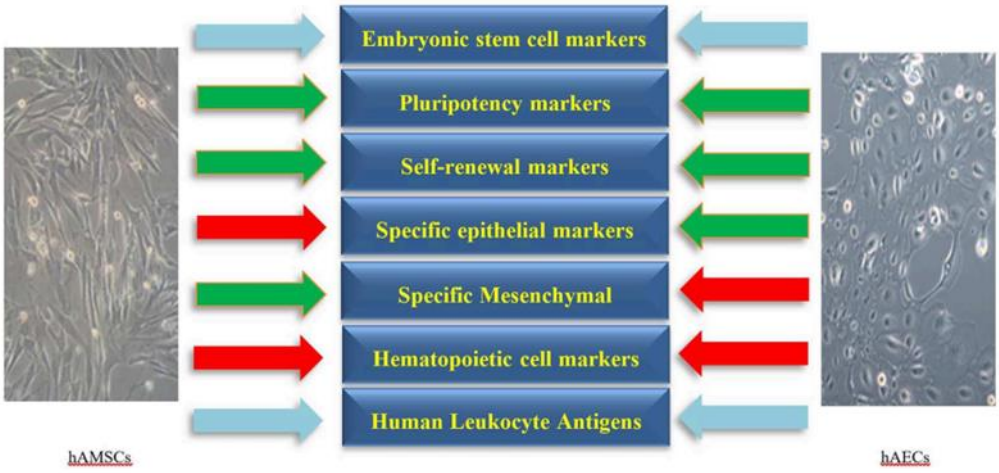

Figure 3. The main negative (red arrows) and positive (green arrows) markers on human amniotic epithelial (hAECs) and mesenchymal stromal cells (hAMSCs). The expression of Human Leukocyte Antigens and embryonic stem cell markers on hAECs and hAMSCs is variable (sky-blue arrows) [55].

2.2 Angiogenesis

Several cytokines were detected in amnion derived cellular cytokines including PDGF, VEGF, angiogenin, TGF-ß2 and TIMP1 and 2[56]. Furthermore, it has shown that amnion derived stem cells secret additional angiogenic factors such as HGF, IGF-1, EGF, HB_EGF and bFGF [46] All these characterized cytokines are associated with angiogenesis which is the first step that occur to repair injured tissue [57] thus, these characterized cytokines may be beneficial in healing acute and chronic wounds[56]. in (2013) investigators have demonstrated that amnion dorsal side stimulates angiogenesis, whereas the epithelial side inhibits the process [58], taken together these results suggest that the angiogenesis potential exhibited by amnion stromal side is dependent on the extracellular matrix (ECM) components which possibly contribute to the production of angiogenesis microenvironment [59]. Beside the important role of amnion ECM,other investigators have studied in detail the function of derived stem cells in promoting healing of injured tissue,[60] were assessed the benefits of human epithelial cells transplantation through subcutaneous injection to improve the wound healing rate of stage iii PUS in rat, but hAEcs were incorporated into the endothelial layer of the arterial wall but did not improve vascular function following balloon injury of the rat carotid artery in rats [61].

There is great controversy on hAMC ability to differentiate into endothelial lineage, although treatment of hAMC with specific media which contain angiogenesis growth factors the cells changed their fibroblast like- morphology and acquired an endothelial–like network but they did not express mature endothelial cell marker vWF and VE-Cadherin[49].In contrast to this result, hAMC–sorted population (hAMC-SP) cultured under hypoxia condition differentiated into endothelial lineage and this may be attributed to upregulation of the gene expression associated with angiogensis through activation of HIS[62]. However, according to some reports conditioned media (CM) collected from hAMc and hAEC possess a survival – enhancing effects on human endothelial cells and this can explain at least in part angiogenic action of amnion derived stem cells. Therefore results of preclinical investigation has demonstrated role of hAEC to reduce vascular stiffening in mice with induced hypertension[63] , Mesenchymal stem cells release several molecules and extracellular vehicles responsible for their therapeutic mechanism of actions. Therefore, several strategies were adopted for the development of a cellular therapeutic intervention through enhancing the release of paracrine factors that have both local and distance effects such as hypoxia,pro-inflammatory stimuli and 3-Dimensional growth [64] together with genetic modification, physiological and pharmacological preconditioning and biomedical based approach represent selective methods to enhance the release of

angiogenic,mitogenic, anti- inflammatory, anti-apoptotic and anti- oxidative effects of Msc[65]. Administration of hAMC-CM can exert beneficial paracrine effects on infarct rat heart with reduced infarct size, attenuated cardiomyocytes apoptosis and ventricular remodeling and stimulated angiogenesis at the infarct border zone [66]. These observations suggest that paracrine factors released by hAMC play a crucial role in the angiogenic potential exhibited by amnion derived mesenchymal cells [67], on the other hand there is no great difference in this property between hAMSc and MSCs derived from different sources such as bone marrow, adipose tissue and umbilical cord blood as well as further studies are required to assess whether hAEC possess this property like hAMSc [68]. [46] Demonstrated that both hAMSc and MSCs showed differences in their soluble factor secretion and angiogenic functions, but these cells could be ideal cell sources for regenerative medicine.

2.3 Neural differentiation potential of amnion derived stem cells

Stem cells isolated from AM have shown immunophenotypes similar to ESCs in addition to their differentiation potential into cells of the three germinal layers. For instance, several studies were carried out to detect the differentiation ability of AECs into neural cells and some growth factors have been suggested to induce the differentiation of AECs into nerve cells such as, basic fibroblast growth factor (bFGF), Epidermal growth factor (EGF), Noggin and Retinoic acid(RA) and the results of immunophenotype detection showed the ability of stimulated cells to express neural markers such as Otx2,TBR2, ß- tubulin III, neuro specific enolase (NSE)andNeuN [20,69]. Thereafter,AECs directed to differentiate into Schwann-like cells by an in vitro co-culture system with Schwann cells derived from rat sciatic nerve, also immunofluorescence assay revealed that AECs subjected to differentiation exhibited positive expression for Schwann cells marker (S100)[70]. Moreover, the neural characteristics of AECs are not restricted to differentiation into neural-lineage, but include the ability of AECs to release neurotrophic factors such as EGF and bFGF(FGF-2) or a novel isoform of these growth factors that maintain survival and stimulate differentiation of chicken neural retinal cells [71] as well as brain derived neurotrophic factor (BDNF) and neurotrophin -3[72]. Beside these features, AECs express markers which are known to be specific for neural lineage for instance microtubule-associated protein-2, glial fibrillary acidic protein and nestin [73,16,74] and possess immunosuppressive property in addition to migration toward inflamed sites in the central nervous system.Generally,these characteristics suggest that AECs could play a crucial role in the treatment of neural disorders such as multiple sclerosis (MS) [75] and Parkinson's disease[72].As well as these cells can promote neural cell survival and regeneration, repair affected neurons, and reestablish damaged neural connections. It is suggested that hAECs may be the most promising candidate for cell-based therapy of neurological diseases [76].

Accumulating evidence suggests that the capability of AMScs to differentiate toward neuronal lineage and express neuronal specific markers [77,78, 16, 17] and under certain culture conditions,hAMSc acquired neurogenic morphology,immunophenotype detection indicated positive expression of S100 and NSE[79]and gene analysis emphasized the results, as the differentiated cells exhibited high levels of expression of neuron associated genes for example NSE,neurofilament–medium, ß- tubulin isotype III(TUJI)and GFAP[78]. The differentiation protocols were performed by treatment the cells with growth factors like EGF, bFGF and RA [79].Several studies have reported that the same growth factors can induce neuronal differentiation of MSCs isolated from hBM-MSc [80], ASCs [81]and rat BM-MSc [82].

Like other types of stem cells[83], AMSCs are assumed to be impacted by environmental cues, as AMSCs expressed nerve-like cells morphology when treated by mouse brain medium and immunofluorescent assay emphasized positive Map-2 expression in AMSCs after treatment and this property besides anti-inflammatory effects, secretion of neurotrophic factors and neuronal differentiation potential suggest that AMSCs would be a suitable candidate for the treatment of nervous system disorders [84].

Although AMSCs possess neural characteristics, groups of researchers focused on the AMSCs immunomodulatory property and their results demonstrated that hAMSCs inhibit the proliferation of purified T- lymphocytes [85].Moreover, the immunomodulatory properties of stem cells derived from AM have been discussed in detail to explain whether these properties depend on cell to cell contact or secretion of specific molecules that exert immunosuppressive activity and according to the literatures reported this property of AM derived stem cells, it became obvious that the therapeutic effects of these cells are attributed to secretion of molecules with trophic and anti-inflammatory effects[86,87,88]. Subsequently, investigators have succeeded to induce AMSCs derived from explant culture of AM into neurospheres which may employed on In ViVo neurodegenerative model [89].

3. CONCLUSIONS

Amnion derived stem cells (epithelial and mesenchymal) possess similar neural characteristics regardless of their morphology and embryonic origin, Furthermore autocrine and paracrine characteristics of these cells can be explored as a cell free therapy in treatment of various degenerative disease specially, central nervous system disorders and wound healing due to crucial role of AESCs and AMSCs in angiogenesis .

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Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

[1] M . Mimeault, and S. K. Batra, "Concise review: recent advances on the significance of stem cells in tissue regeneration and cancer therapies", Stem cells, vol.24, no.11, 2319-2345, 2006, DOI: https://doi.org/10.1634/stemcells.2006-0066 .

[2] Ch. C.Ude, A, Miskon, R. B. Idrus, and M. Bin Abu Bakar, "Application of stem cells in tissue engineering for defense medicine", 2018, DOI: https://doi.org/10.1186/s40779-018-0154-9 .

[3] N. H. Kang, K. A. Hwang, S.U. Kim, Y. B. Kim , S. H. Hyun, E. B. Jeung, and K. C. Choi, "Potential antitumor therapeutic strategies of human amniotic membrane and amniotic fluid-derived stem cells", Cancer gene therapy, vol. 19, no. 8, 517-522, 2012, DOI: https://doi.org/10.1038/cgt.2012.30 .

[4] L. R. kass, EH. Blackburn, R. S. Dresser, D.W. Foster, F. Fukuyama, M. S.Gazzanig, R. P. Georg, A. Gomezobo,W. B. Hurlbut, C. Krauthammer, W. F. May, P. McHuch, G. C. Meilander, J. D. Rowley, M. J. Sande, and J. Q.Wilson, "a report of the presidents council on bioethics", 414Pp, 2004.

[5] J. Panno, "Stem cell research: Medical applications and ethical controversy", Infobase Publishing, 2014.

[6] C. Pipino, P. Shangaris, E. Resca, S. Zia, J. Deprest, N.J. Sebire, and P. De Coppi, "Placenta as a reservoir of stem cells: an underutilized resource", British medical bulletin, vol.105, no. 1, 2013, DOI: 10.1093/bmb/lds033 .

[7] I. L.Weissman, "Stem cells: units of development, units of regeneration, and units in evolution", cell, vol.100, no. 1, 157-168, 2000.

[8] W. Wagner, F. Wein, A. Seckinger, M. Frankhauser, U. Wirkner, U. Krause, and A. D. Ho, "Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood", Experimental hematology, vol.33, no. 11, 1402-1416, 2005, DOI: https://doi.org/10.1016/j.exphem.2005.07.003 .

[9] B. R. Sousa, R. C. Parreira, E. F. Fonseca, M. J. Amaya, F. M. Tonelli, S. M. Lacerda, and R.R. Resende, "Human adult stem cells from diverse origins: an overview from multiparametric immunophenotyping to clinical applications", Cytometry Part A,vol. 85, no. 1, 43-77, 2014, DOI: https://doi.org/10.1002/cyto.a.22402 .

[10] D. Jiang, and K. Scharffetter-Kochanek, "Mesenchymal stem cells adaptively respond to environmental cues thereby improving granulation tissue formation and wound healing", Frontiers in cell and developmental biology, vol.8, 697 , 2020, DOI: https://doi.org/10.3389/fcell.2020.00697 .

[11] Z. Golipoor, R. KashaniI, M. Akbari , G. Hassanzadeh, F. Malek, and Mahmoudi, "Differentiation of adipose-derved stem cells into Schwann cell phenotype in comparison with bone marrow stem cell", I. J, B. M.S, vol.13, no. 3, 76- 84, 2010

[12] K. Kadar, M. Kiraly, B. Porcssalmy, B. Molnar, G. Z. Racz, J. Blazsek, K. Kallo, E. L.S zabo, I. Gera, G. Gerber, and G. Varga, "Differentiation potential of stem cells from human dental origin-promise for tissue engineering", J. Phsiol. Pharmacol, vol.60, no.167- 176, 2009.

[13] Y. Han, X. Li, Y. Zhang, Y. Han, F. Chang, and J. Ding, "Mesenchymal stem cells for regenerative medicine", Cells, vol.8, no. 8, 886, 2019, DOI: https://doi.org/10.3390/cells8080886 .

[14] E. Zomorodian, and M. Eslaminejad,"Mesenchymal stem cells as a potential source for bone regeneration", Stem Cells international, 9 Pp, 2012.

[15] O. Parolini, F. Alviano, G. P. Bagnara, G. Bilic, H. J. Bühring, M. Evangelista, and S.C. Strom, "Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells", Stem cells, Vol.26, no. 2, 300-311, 2008, DOI: https://doi.org/10.1634/stemcells.2007-0594 .

[16] C. L. Insausti, M. Blanquer, P. Bleda, P. Iniesta, M. Majado Martínez, G. Castellanos Escrig, and J.M. Moraleda Jiménez, "The amniotic membrane as a source of stem cells", Histology and histopathology, 2010.

[17] G. Kmiecik, W. Niklinska, P. Kuc, J. Pancewicz- Wojtkiewicz, D. Fil, A. Karwowska, J. Karczewski, and Z. Mackiewicz, " Fetal membrane as a source of stem cells". Adv. Med. Sci.,vol. 58, no. 2, 185- 195, 2013.

[18] M. Witkowska-Zimny, and E. Wrobel, "Perinatal sources of mesenchymal stem cells: Wharton's jelly, amnion and chorion", Cellular and Molecular Biology Letters, vol.16, no. 3, 493-514 , 2011, DOI: https://doi.org/10.2478/s11658-011-0019-7 .3

[19] A. Kwon, Y. Kim, M. Kim, J. Kim ,H. Choi , D. W. Jekari , S. Lee , J. M. Kim, J. C.Shin, and I.Y. Park , "Tissue – specific differentiation potency of mesenchymal stromal cells from perinatal tissues", Nature, 6, 2016, DOI: https://doi.org/10.1038/srep23544 .

[20] H. Niknejad, H. Peirovi, A. Ahmadiani, J. Ghanavi, and M. Jorjani, " Differentiation factors that influence neuronal markers expression in vitro from human amniotic epithelial cells", Eur Cell Mater, Vol.19, 22-29 , 2010

[21] J. A. Brazzo, "A comprehensive reviewof the amniotic membrane and amniotic fluid", Master thesis, School of medicine, Boston university, pp112, 2014

[22] B. Farhadi hossein abadia, M. Farahania, T.Tayebia, A. Jafaria, F. Biniazana, K. Modaresifarc, H. Moravvejd, S. Bahramie, H. Redle, L.Tayebif, and H. Niknejada, "Amniotic membrane and its epithelial and mesenchymal stem cells as an appropriate source for skin tissue engineering and regenerative medicine", Artificial Cells, Nanomedicine and Biotechnology, 46, S431–S440, 2018, DOI: https://doi.org/10.1080/21691401.2018.1458730 .

[23] F. Alviano, V. Fossati, C. Marchionni, M. Arpinati, L. Bonsi, M. Franchina, G. Lanzoni, S. Catoni, C. Cavallini, F. Bianchi, P. L.Tazzari, G. Pasquinelli, L. Foroni, C.Ventura, A. Grossi and G. P. Bagnara, "Term amniotic membrane is a highthroughput for multipotentmesenchymal stem cells with the ability to differentiate into endothelial cells in vitro*", BM C Dev Biol*,vol. 7, 14Pp, 2007.

[24] S.Takashima, H. Ise, P.Zhao, T. Akaike and T. Nikaido, "Human amniotic epithelial cells possess hepatocyte-like characteristics and functions", Cell Structure and Function, vol.29, no, 73-84, 2004, DOI: https://doi.org/10.1247/csf.29.73

[25] A. Lindenmair, T. Hatlapatka, G. Kollwig, S. Hennerbichler, C. Gabriel, S. Wolbank, and C. Kasper, "Mesenchymal stem or stromal cells from amnion and umbilical cord tissue and their potential for clinical applications", Cells, no.1, vol. 4, 1061-1088, 2012, DOI: https://doi.org/10.3390/cells1041061

[26] D. Asl K, H. Shafaei, J. S. Rad, and H. Nozad, "Comparision of characteristics of human amniotic membrane and human adipose tissue derived mesenchymal stem cells", W.J PS., vol.6, no. 1, 33-39, 2017.

[27] H. Wegmeyer, A. M. Bröske, M. Leddin, K. Kuentzer, A. K. Nisslbeck, J. Hupfeld and M. Neubauer, M. , "Mesenchymal stromal cell characteristics vary depending on their origin", Stem cells and development, vol. 22, no.19, 2606-2618, 2013, DOI: https://doi.org/10.1089/scd.2013.0016 .

[28] G. L. Bourne, "The microscopic anatomy of the human amnion and chorion",1959.

[29] S. Ilancheran, A. Michalska, G. Peh, E.M. Wallace, M. Pera, and U. Manuelpillai, " Stem cells derived from human fetal membranes display multilineage differentiation potential", Biology of Reproduction, vol.77, no.3, 577-588, 2007, DOI: https://doi.org/10.1095/biolreprod.106.055244

[30] H. Niknejad, H. Peirovi, M. Jorjani, A. Ahmadiani, J. Ghanavi, and A. M. Seifalian, "Properties of the amniotic membrane for potential use in tissue engineering" Eur Cells Mater, vol.15, 88-99, 2008.

[31] T. Miki, and S. C. Strom, "Amnion-derived pluripotent/multipotent stem cells", Stem cell reviews, vol.2 ,no.2, 133-141, 2006, DOI: https://doi.org/10.1007/s12015-006-0020-0 .

[32] S. Díaz-Prado, E. Muiños-López, T. Hermida-Gómez, M.E. Rendal-Vázquez, I. Fuentes-Boquete, F. J. De Toro, and F. J. Blanco, "Multilineage differentiation potential of cells isolated from the human amniotic membrane", Journal of Cellular Biochemistry, vol.111, no.4, 846-857, 2010, DOI: https://doi.org/10.1002/jcb.22769 .

[33] R. Iwasaki, S. Matsubara, T. Takizawa, and T. Takayama, "Human amniotic epithelial cells are morphologically homogeneous: enzymehistochemical, tracer, and freeze-substitution fixation study", European Journal of Histochemistry, vol.47, no.3, 223-232, 2003, DOI: https://doi.org/10.4081/831 .

[34] J. D. Aplin, S. Campbell, and T. D. Allen, "The extracellular matrix of human amniotic epithelium: ultrastructure, composition and deposition", Journal of Cell Science, vol.79, no.1, 119-136, 1985, DOI: https://doi.org/10.1242/jcs.79.1.119 .

[35] A. Chopra, and B. S. Thomas, "Amniotic membrane: a novel material for regeneration and repair", Biomimetics Biomaterials and Tissue Engineering, vol.18, 8Pp, 2013.

[36] F. Chehelcheraghi, H. Eimani , S. Homayoonsadraie, G.Torkman, A. Amini , H. A. Majd, and H. Shemshadi, "Effects of acellular amniotic membrane matrix and bone marrow –derived mesenchymal stem cells in improving random skin flap survival in rats", Iran Red Crescent Med. J., vol.18, no.6, 11Pp, 2016, DOI: https://doi.org/10.3390/pharmaceutics15030748 .

[37] I. Fathi, and T. Miki, "Human amniotic epithelial cells secretome :components, bioactivity, and challenges", Frontiers in Medicine, vol. 8, 763141, 2022, DOI: https://doi.org/10.3389/fmed.2021.763141

[38] A.W. Mazgalova, R. Todorov, K. Sterava, and W. Trencheva, "Use of amniotic membranes as biological dressings in contemporary treatment of burns", Ann.Medit. Burns Club., VII , no.4, 5P, 1994.

[39] M. D. S. Rahman, R. Islam, M. M. Rana, L. S. Spitzhorn, S. M. Rahman, J. Adjaye, and S. M. Asaduzzaman, "Characterization of burn wound healing gel from human amniotic membrane and Alo vera extract", BMC Complmentary and Alternative Medicine, vol.19, 115, 2019, DOI: https://doi.org/10.1186/s12906-019-2525-5 .

[40] H. Niknejad, M. Khayat-Khoei, H. Peirovi, and H. Abolghasemi, "Human amniotic epithelial cells induce apoptosis of cancer cells: a new anti-tumor therapeutic strategy", Cytotherapy, vol.16, no.1, 33-40, 2014, DOI: https://doi.org/10.1016/j.jcyt.2013.07.005 .

[41] M. Tabatabaei, N. Mosaffa, S. Nikoo, M. Bozorgmehr, R. Ghods,S. Kazemnejad, S. Rezania, B. Keshavarzi, S. Arefi, F. Ramezani-Tehrani, E. Mirzadegan, and A. H. Zarnani, "Isolation and partial characterization of human epithelial cells : the effect of trypsin", Avicenna Journal of Medical Biotechnology, vol. 6, no.1, 10-20, 2014.

[42] B. Motamedi, T. A. T. Ibrahim, B. AbulA, Z. N. Allaudin, M. Moshrefi, Hajghani, and S. N. NematollahiMahan, "Characteristics of human amniotic epithelial cells and bone marrow mesenchymal stem cells in a parallel study, Hanging drop colony formation and doubling time", J. Regen. Med., vol.4, 1000120, 2015.

[43] B. Okere, F. Alviano, R. Costa, D. Quaglino, F. Ricci, M. Dominici, and L. Iughetti, "In vitro differentiation of human amniotic epithelial cells into insulin-producing 3D spheroids", International Journal of Immunopathology and Pharmacology, vol.28, no.3, 390-402, 2015, DOI: https://doi.org/10.1177/0394632015588439 .

[44] T. Miki, T. Lehmann, H. Cai, D. B. Stolz, and S.C Strom, " Stem cell characteristics of amniotic epithelial cells" Stem Cells, vol.23, no.10, 1549-1559, 2005, DOI: https://doi.org/10.1634/stemcells.2004-0357 .

[45] C. Xu, W. Ahmed, L. Xie, Y. Peng, Q. Wang, and M. Guo, "Producing Human Amniotic Epithelial Cells-only Membrane for Transplantation", Bio.R., xiv, 837377, 2019, DOI: https://doi.org/10.1101/837377

[46] Q.Wu, T. Fang, H. Lang, M. Chen, P. Shi, X. Pang, and G. Qi, "Comparison of the proliferation, migration and angiogenic properties of human amniotic epithelial and mesenchymal stem cells and their effects on endothelial cells", International Journal of Molecular Medicine, vol.39, no.4, 918-926, 2017, https://doi.org/10.3892/ijmm.2017.2897 .

[47] M. G. Roubelakis, Q. Trohatou, and N. P. Anagnou, "Amniotic fluid and amniotic membrane stem cells: marker discovery", Stem Cells Int., 9Pp, 2012.

[48] G. Pratama , V. Vaghjiani , J. Y. Tee ,Y. H. Liu , J. Chan, C. Tan, P. Murthi, C. Gargett, And U. Manuelpillai, "Changes in culture expanded human amniotic epithelial cells: implications for potential therapeutic applications", Plos One , 6 Issue 11, e26136, 2011.

[49] J. Konig, "Endothelial differentiation potential of amnion – derived mesenchymal stromal cells", Dissertation, Institute of Cell Biology, Histology and Embryology, Medical University of Graz: 67Pp, 2011.

[50] G. Pasquinelli, P. Tazzari, F. Ricci, C. Vaselli, M. Buzzi, R. Conte, and E. Lucarelli, "Ultrastructural characteristics of human mesenchymal stromal (stem) cells derived from bone marrow and term placenta" Ultrastructural Pathology,vol. 31, no1, 23-31, 2007, DOI: https://doi.org/10.1080/01913120601169477 .

[51] H. L. Huang, H.w. Hsing, T. C. Lai, T. W.Chen, T. R. Lee, H. T. Chan, P. C. Lyu, C. L.Wu , Y. C. Lu, S. T. Lin, C. W. Lin, C. H. Lai, H. T. Chang, H. C. Chou, and H. L. Chang, "Trypsin- induced proteome alteration during cell subculture in mammalian cells", J. Biomed. Sci., vol.17, 2010,

[52] R. Wagey, "Mini-review mesenchymal cells ",Stem cell Technologies", 2008.

[53] E. M. Horwitz , K. L. Black, M. Dominici, I. Mueller, I. Slaper-Cortenbach, F. C. Marini, R. J. Deans, D. S. Krause, and A. Keating, "Position paper clarification of the nomenclature for MSC : The international society for cellular therapy position statement", Cytotherapy, vol. 7,- 393-395, 2005.

[54] M. Dominici, K. L. Blanc, I. Mueller, I. slaper-Cortenbach, F. C. Marini, D. S. Krause, R. J. Dean, A. Keating, D. J. Prockop, and E. M. Horwitz, "Position paper minimal criteria for defining multipotentmesenchymal stromal cells.The international society for cellular therapy position statement", Cytotherp.,vol. 8, 315-317, 2006.

[55] S. H.Ghamari, M. Abbasi- Kangevari, T. Tayebi, S. Bahrami, and H. Niknejad, "The bottlenecks in translating placenta -derived amniotic epithelial and mesenchymal stromal cells into the clinic :current discrepancies in marker reports", Frontiers in Bioengineering and Biotechnology, vol.8, 13p, 2020.

[56] D. L. Steed, C, Duffy, C. Smith, V. Marshall, R. Rupp, and M. Robson, "Amnion – derived cellular cytokine solution", Journal of Plastic Surgery, vol.8, 157- 165, 2008.

[57] V. Kumar, A. K. Abbas, N. Fausto, and R. N. Mitchell, Robbins basic pathology, 8th ed.Saunders Elsevier,: Xiii+ 903, 2007.

[58] H. Niknejad, G. Paeini-Vayghan, F. A. Tehrani, M. Khayat-Khoei, and H. Peirovi, "Side dependent effects of the human amnion on angiogenesis", Placenta, vol.34, no.4, 340-345.**2013,** DOI: https://doi.org/10.1016/j.placenta.2013.02.001 .

[59] S. N. Hashim, M. F. H. Yusof, W. Zahari, K. B. A. AminNoordin, T. P. Kannan, S. S. Abdul Hamid, K. I. Mokhtar, and A. Ahmad, "Angiogenic potential of extracellular matrix of human amniotic membrane", Tissue Eng. Med., vol.13, no. 3, 211-217, 2016, DOI: https://doi.org/10.1007/s13770-016-9057-6 .

[60] A. Zhou, X. Zheng, L. Yu, M. Quan, X. Shao, and Z. Jiang, "Mechanisms of human amniotic epithelial cell transplantation in treating stage III pressure ulcer in a rat model", Experimental and Therapeutic Medicine, vol.10, no.6,2161-2168, 2015, DOI: https://doi.org/10.3892/etm.2015.2778 .

[61] G. Vácz, A. Cselenyák, Z. Cserep, R. Benkő, E. Kovacs, E. Pankotai and Z. Lacza, "Effects of amniotic epithelial cell transplantation in endothelial injury", Interventional Medicine and Applied Science, vol.8, no. 4, 164-171, 2016, DOI: https://doi.org/10.1556/1646.8.2016.4.6 .

[62] N. Maruyama, K. Kokubo, T. Shinbo, M. Hirose, M. Kobayashi, N. Sakuragawa, and H. Kobayashi, "Hypoxia enhances the induction of human amniotic mesenchymal side population cells into vascular endothelial lineage", International Journal of Molecular Medicine, vol.32, no.2, 315-322 ,2013, DOI: https://doi.org/10.3892/ijmm.2013.1412 .

[63] Q. N. Dinh, C. Lo, D. W. Zhang, V. Vivian Tran, T. Gibson-Hughes, H. Sherif,A. Diep, H. A. Kim, Shenpeng, S. R. Zhang, J. Liz, L. J. Barreto-Arce1, M. Jelinic, A. inh, T. V. Arumugam, S. T. Chan, R. Rebecca Lim, R. Grant, G. R. rummond, G. Christopher, C. G. Sobey, and T. M. Michael De Silva, "Human amnion epithelial cell therapy reduces hypertension-induced vascular stiffening and cognitive impairment", Scientific Reports,vol. 14, 1837, 2024, DOI: https://doi.org/10.1038/s41598-024-52214-0

[64] M. Madrigal, K. S. Rao, and N. H. Riodan, "A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods", J. Transl. Med., vol.12, 14Pp, 2014, DOI: https://doi.org/10.1186/s12967-014-0260-8 .

[65] D. Pankajakshan, and D.K. Agrawal, "Mesenchymal stem cell paracrine factors in vascular repair and regeneration", J. Biomed. Technol.. Res., 1, 9Pp, 2014, DOI: https://doi.org/10.19104/jbtr.2014.107 .

[66] P. Danieli, G. Malpasso, M. C. Ciuffreda, E. Cervio, L. Calvillo, F. Copes, and M. Gnecchi, "Conditioned medium from human amniotic mesenchymal stromal cells limits infarct size and enhances angiogenesis", Stem Cells Translational Medicine, vol.4, no. 5, 448-458, 2015, DOI: https://doi.org/10.5966/sctm.2014-0253 .

[67] S.Wu, S. Sun, W. Fu, Z. Yang, Z. Yao,Z. and Z. Zhang, "The Role and Prospects of Mesenchymal Stem Cells in Skin Repair and Regeneration", Biomedicine, vol. 12,743, 2024, DOI: https://doi.org/10.3390/biomedicines12040743 .

[68] C. H. Fang, J. H. Joe, Y. S. Song, B. I. So, S. M. Lim, and K. S. Kim, "In vivo differentiation of human amniotic epithelial cells into cardiomyocyte-like cells and cell transplantation effect on myocardial infarction in rats: comparison with cord blood and adipose tissue-derived mesenchymal stem cells", Cell transplantation, vol. 21, no.8, 1687-1696, **2012**, DOI: https://doi.org/10.3727/096368912X653039 .

[69] I. L.García-Castro, G. García-López, D. Ávila-González, H. Flores-Herrera, A. Molina-Hernández, W. Portilloand N. F. Díaz, "Markers of pluripotency in human amniotic epithelial cells and their differentiation to progenitor of cortical neurons", PloS one, vol.10, no.12, e0146082, 2015, DOI: https://doi.org/10.1371/journal.pone.0146082 .

[70] S. Zhu, J. Li, Q. Zhu, T. Dai T, B. X. Z. Zho, J. Xiang, and X. Liu, "Differentiation of human amniotic epithelial cells into Schwann- like cells via indirect co- culture with Schwann cells in vitro", Molecular Medicine Reports, vol.11, 1221- 1227, 2015, DOI: https://doi.org/10.3892/mmr.2014.2881 .

[71] S. Venkatachalam, T. Palaniappan, P. K. Jayapai, S. NeelaAmegan, S. S. Rajan, and V. P. Muthian, "Novel neurotrophic factor secreted by amniotic epithelial cells", Bio Cell, vol, 33, no. 2, 81-89, 2009.

[72] J. A. Pawitan, "Prospect of cell therapy for Parkinson's disease", Anatomy and Cell Biology, vol. 44, no4, 256, 2011, DOI: https://doi.org/10.5115/acb.2011.44.4.256 .

[73] A. Toda, M. Okabe, T. Yoshida, and T. Nikaido, "The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues", Journal of Pharmacological Sciences, vol.105, no.3, 215-228, 20, 2007, DOI: https://doi.org/10.1254/jphs.CR0070034

[74] Z. Wu, G. Hui, , Y. Lu, T. Liu, Q. Huang, and L. Guo, "Human amniotic epithelial cells express specific markers of nerve cells and migrate along the nerve fibers in the corpus callosum", Neural Regeneration Research, vol.7, no. 1, 41-45, 2012, DOI: 10.3969/j.issn.1673-5374.2012.01.007.

[75] C. McDonald, C. Siatskas, and C. C. Bernard, "The emergence of amnion epithelial stem cells for the treatment of multiple sclerosis", Inflammation and Regeneration,vol. 31, no. 3, 256-271, 2011, DOI: https://doi.org/10.2492/inflammregen.31.256 .

[76] H. Xu, J. Zhang, K. S. Tsang, H. Yang, and W. Q. Gao, "Therapeutic potential of human amniotic epithelial cells on injuries and disorders in the central nervous system", Stem cells International, vol.2019, no. 1, 5432301, 2019, DOI: https://doi.org/10.1155/2019/5432301 .

[77] C. B. Portmann-Lanz, A. Schoeberlein, A. Huber, R. Sager, A. Malek, W. Holzgreve, and D.V. Surbek, "Placental mesenchymal stem cells as potential autologous graft for pre-and perinatal neuroregeneration", American journal of obstetrics and gynecology, Vol. 194, no.3, 664-673 , 2006, DOI: https://doi.org/10.1016/j.ajog.2006.01.101 .

[78] T. Tamagawa, I. Ishiwata, H. Ishikawa, and Y. Nakamura, "Induced in vitro differentiation of neural-like cells from human amnion-derived fibroblast-like cells", Human cell, olv.21,no,2, 38-45, 2008, DOI: https://doi.org/10.1111/j.1749-0774.2008.00049.x .

[79] D. M. Pop, O. Soritau,S. Susman, D. Rus-Ciuca, I. S. Groza, R. Ciortea, D. Mihu, and C. M. Mihu, "Potential of placental- derived human mesenchymal stem cells for osteogenesis and neurogenesis", Rom. J. Morphol. Embryol., vol. 56, no. 3, 989- 996, 2015 .

[80] A. Hermann, R. Gastl, S. Liebau, M. O. Popa, J. Fiedler, B. Boehm, and A. Storch, "Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells", Journal of Cell Science, vol. 117, no.19, 4411-4422, 2004, DOI: https://doi.org/10.1242/jcs.01307

[81] R. Taran, M. K.Mamidi, G. Singh, S. Dutta, L. S. Parhar, J. P. John, and A. K. Das, "In vitro and in vivo neurogenic potential of mesenchymal stem cells isolated from different sources", Journal of Biosciences, vol. 39, no. 1, 157-169 , 2014, DOI: https://doi.org/10.1007/s12038-013-9409-5

[82] A. A. Mohammed Karim, I. N. Waheed, and M. A. Gali, "In Vitro Differentiation of Mesenchymal Stem Cells Derived from Rat Bone Marrow into Nerve-Like Cells", International Journal of Pure and Applied Sciences and Technology,vol. 27, no.1, 2015.

[83] Y. S. Choi, Y. B. Park,C. W. Ha, J. A. Kim, J. C. Heo, W. J. Han, S. Y. Oh, and S. J. Choi, "Different characteristics of mesenchymal stem cells from different layer of full term placenta", Plos One, 0172642, 2017, DOI: https://doi.org/10.1371/journal.pone.0172642 .

[84] S. Shahraki, H. Jalali, K. Parivar, N. H. Roudbari, M. Nabiuni, and Z. Heidari, "Neuronal differentiation of mouse amnion membrane derived stem cells in response to neonatal brain medium", J. Cell Mol. Res.,vol. 6, no. 2, 76-82, 2014, DOI: https://doi.org/10.14677/jcmr.v6i2.37591 .

[85] F. Alikarami, F. Yari, N. Amirzadeh, M.Nikougoftar, and M. A. Jalili, "The immunosuppressive activity of amniotic membranemesenchymal stem cells on T lymphocytes", Avicenna J. Med. Biotechnol., vol.7, no. 3, 90- 96, 2015.

[86] G. Bilic, S. F. Zeisberger, A. S. Mallik, R. Zimmmermann, and A. H. Zisch, "Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy", Cell Transplant., vol.17, 955- 968, 2008.

[87] C. L. Insausti, M. Blanquer, A. M. Garcia- Hernandez, G. Castellanos, and J. M. Moraleda, "Amniotic membrane –derived stem cells: immunomodulatory properties and potential clinical application", Stem Cells and Cloning, Adv. Appl. , vol. 7, 53- 63, 2014 .

[88] H. G. Carvajal, P. Suarez-Meade, and C. V. Borlongan, "Amnion- derived stem cell transplantation: Anovel treatment for neurological disorders", Brain Circulation, 2, 1-7, 2016, DOI: https://doi.org/10.4103/2394-8108.178537 .

[89] Ö. Ö, Akkaya, A. S. M. G. Kibria, A. Yagci, M. Erdogan, and K. Altunbas, "Explant cuture and multilineage differentiation of amniotic membrane derived stem cells", Ankara Univ. Vet. Fak. Derg.,vol. 69, 191- 199, 2022.