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Research Article

Differentiation and Structural Investigation of Bone Marrow Stem Cell-derived Retinal Pigment Epithelium Cells

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Abstract

Background: To investigate the ultrastructure of retinal pigment epithelium (RPE) derived from rat bone marrow stromal stem cells (BMSCs).

Materials and methods: In this study, mesenchymal stem cells were extracted from the bone marrow of rats. They were then cultured in Transwell plate containing RPE differentiation cocktail and cultured for sixty days. Immunocytochemistry investigation was performed on the bone marrow cells and RPE cells. To evaluate the melanin pigment as well as the apical microvilli, tight junction of differentiated RPE cells, hematoxylin and Eosin staining, scanning and transmission electron microscopy were performed.

Results: Immunocytochemistry investigation of the bone marrow cells indicated that more than 90% of these cells showed mesenchymal stem cells markers. The results also showed that 76.52% of RPE cells indicated RPE65 specific protein, and 62.06% of them revealed tight junction protein (ZO-1). Furthermore, optical microscopy findings indicated that these cells were single-layer with apical villi on top of them. The results of scanning and transmission electron microscopy showed that these cells after 60 days had melanin pigment and apical microvilli as well as tight junction between the cells.

Conclusion: We found that BMSCs can be differentiated into RPE cells and express the specific markers of these cells. We also indicated that the differentiated cells in comparison to normal cells had apical microvilli as well as melanin granules, and there was also cell junction between cells.

Keywords: Bone Marrow Stem Cells; Retinal Pigmented Epithelium; Apical Microvilli; Tight Junction; Melanin; Electron Microscopy

Introduction

Retinal pigment epithelial is a layer of epithelial cells located between the retina and the Bruch's membrane that are responsible for maintaining retinal health. RPE dysfunction causes retinal damage and vision impairment, including AMD and retinitis pigmentosa (RP) [1]. Since there is no current treatment for dry type of macular degeneration, replacement of the lost RPE is one of the therapeutic goals [2].

Overall, RPE can be produced from a variety of cellular sources, including reprogrammed stem cells (iPSCs), embryonic cells (ESCs), and adult stem cells (ASCs) [3-8]. The latest one can be separated from different parts of the body, such as bone marrow and adipose tissue. These cells are safer than the first two cell types and have a very low risk of tumor formation and transplant rejection. ASCs also secrete a variety of growth factors that play a supporting role in damaged tissue. Finally, these cells have the ability to differentiate into other cells in various tissues, including nerve tissues [4,9-12]. In addition to adult stem cells, including bone marrow stem cells (BMSCs), adipose tissue stem cells (ADSCs), umbilical cord cells (UCSCs), ciliary body-derived cells (CBCs), and retinal pigment progenitor cells (RPs) to produce RPE cells there are other cells that cells have recently been considered: human and mouse embryonic stem cells and induced pluripotent stem cells [3,4,13,14].

Studies show that ASCs have the ability to differentiate into RPE in both *in vitro* and *in vivo* [3,15-18]. However, studies on ESCs also show that these cells are able to produce RPE using different protocols and exhibit the characteristics of RPE [3,18-21]. However, there are some limitations regarding the use of these cells, including the possibility of tumor formation, transplant rejection, and ethical issues [22]. To overcome this problem, researchers have studying reprogrammed cells, in which somatic cells are used to create pluripotent cells, then differentiate them into target cells. The researchers also investigated culture-based production of RPEs using this technique [3], but they found these cells, like embryonic stem cells, face problems, including the fact that these cells quickly undergo the aging process after differentiation into RPE [23].

RPE are produced from adult mesenchymal stem cells (MSCs) in a variety of ways. For example, it is possible to generate RPE by transfection or transduction. These cells can also be produced using co-culture methods or small induction molecules [22].

The cells produced must be able to show the characteristics of normal cells, including cell junctions, melanin pigment, apical microvilli and expression of specific proteins. The production of such a cell faces many challenges and, therefore, achieving it is one of the goals of cell therapy for retinal degeneration. Studies show that the direct co-culture method of human bone marrow mesenchymal stem cells (hBMSCs) produces pigmented cells, but these cells do not show the function of RPE, therefore, the use of indirect co-culture of human bone marrow stem cells with swine RPE cells using transwell plates leads to the production of RPEs with phagocytic function, secretion of BDNF and GDNF factors, and production of mature melanin pigment and the emergence of primary microvilli on the surface of cells [22].

In a previous study, we showed that human adipose mesenchymal stem cells (hADSCs) and rat bone marrow stromal stem cells (rBMSCs) differentiate into RPE-like cells [24,25]. But we didn't perform ultrastructure of them. Therefore, the aim of the present study was to slightly change the method by Transwell plate to investigate the cell junctions, melanin pigment, and apical microvilli of the produced pigment cells.

Materials and Methods

Isolation and culture of bone marrow's stem cells

Male hooded rats (Razi Institute, Iran, Tehran), weighing 250-300 g were euthanized under deep anesthesia (ketamine/xylazine at 500/50 mg/kg i.p.). The muscles attached to femur and tibia were removed, and the bone marrow was extracted using a 5.0 ml syringe, then seeded (0.7×10^6) into T-25 flask. Next, DMED/F12 medium and FBS 10% was added to it, and cultured in incubator with 5% CO2, humidity of 95%, and temperature of 37°C. After 48 hours, the floating cells were separated, new medium was added to the floor-attached cells, and culture medium of cells was replaced once every 48 hours.

Production of retinal pigmented epithelium

Differentiation of BMSCs into RPE cells, was performed, as previously described with some modification [25]. BMSCs in third passage lifted by trypsin 0.025% and following centrifuge (1500 rpm, 5 min, RT) seeded (0.1×10^6) in Transwell plate (Corning co.) and differentiated in two stages. Initially, they were placed in DMEM/F12 medium containing N2 supplement and 20 ng/ml of basic fibroblast growth factor (bFGF; bFGF; Chemicon, Hofheim, Germany), and after two days, the medium was changed to DMEM medium with FBS 1%, 2 mM L-glutamine (Sigma Aldrich), 100 units/ml penicillin, 100 µg/ml streptomycin, 10^{-11} M triiodothyronine (Sigma Aldrich, CAS Number 6397, MW.:672.96, Steinheim, Germany), 2.4×10^{-6} M Putrescin (Sigma Aldrich, CAS Number 5780, FW.:161.07, Steinheim, Germany), 6.3×10^{-8} M Transferrin (Sigma Aldrich, CAS Number 8158, MW.:76-

81, Steinheim, Germany), 8.3 × 10^{-7} M insulin (Sigma Aldrich, CAS Number 16634, FW.:5733.5, Steinheim, Germany), 3.7 × 10^{-7} M Linoleic acid (Sigma Aldrich, CAS Number L1012, FW.:-280.45, Steinheim, Germany), 2.8 × 10^{-8} M Hydrocortisone (Sigma Aldrich, CAS Number H0888, FW.:362.46, Steinheim, Germany), 1.4 × 10^{-8} M Selenous acid (Sigma Aldrich, CAS Number 211176, FW.:128.97, Steinheim, Germany) were used. The cells were cultured in this medium for 6-8 weeks (151-152).

Extraction of normal pigmented cells

To extract native RPE cells as positive control of BMSCs-RPE following procedure was performed. Briefly male hooded rats (Razi Institute, Iran, Tehran), weighing 250-300g were euthanized under deep anesthesia (ketamine/xylazine at 500/50 mg/kg i.p.), and the eyes were enucleated. The eyes washed with BSS solution at pH=8 which contained gentamicin and kanamycin. Thereafter, the eyes were exposed to two enzymatic solutions at 37°C. Initially, they were placed in balanced salt solution (BSS) solution with pH = 7 which contained collagenase and hyaluronidase for 45 to 90 minutes. Next, the eyes were placed inside the second enzymes solution which contained BSS with pH=8 and trypsin 0.1% for 57 to 72 minutes. Once the enzymatic digestion time finished, the eyes were placed inside the culture medium. They were put inside trypsin, then each eye was opened and transferred to MEM culture medium with FPS 20% and L-glutamine 1%. The separated RPE layer was transferred to a 15-cm conical tube and centrifuged (1500 rpm, 5 min, RT). Next, the RPE layer was washed with Calcium- and Magnesium-Free (CMF) solution which had no calcium and magnesium at pH = 8. The RPE layer was again placed inside trypsin 0.1% and incubated for 2 min at 37°C. It was then pipetted so that the cells would be separated from each other as a suspension. The culture medium was added to enzymatic solution and centrifuged, where it was centrifuged for 2 min at 100 rpm. Thereafter, the supernatant was extracted, and eventually transferred to flask after counting by hemocytomer with density 90-160 cells per mm^2.

Immunocytochemistry

For BMSCs immunocytochemistry, the cells at third passage were fixated using paraformaldehyde 4% for 15 minutes. They were then washed with phosphate-buffered saline (PBS) and blocked using rabbit serum blocking solution for one hour at 37°C. Next, the cells were incubated with primary antibodies (Table 1) at 4°C overnight. The negative control was also done with this same method though without using the primary antibody. After washing the primary antibody, the cells were incubated with the secondary antibodies (Table 1) for one hour at room temperature. After washing, the nucleus of cells was counterstained using propidium iodide (PI) and 4′,6-diamidino-2-phenylindole (DAPI) for 5 min. Eventually, the cells were washed and observed using fluorescent microscope. All observations and photography were performed using a fluorescent microscope (BX41; Olympus Corp., Tokyo, Japan) equipped with a digital camera (DP70; Olympus Corp.). Image processing and quantification were performed using a software (Photoshop CS7; Adobe Systems, Mountain View, CA, USA) and image analysis software (ImageJ 1.46r, Wayne Rasband, National Institutes of Health, USA, http://imagej.nih.gov/ij.).

For BMSCs-RPE immunocytochemistry, the cells on Transwell plate were fixated with paraformaldehyde 4% for 15 min, and then were blocked in PBS solution containing 1% bovine serum albumin, 0.1% Triton X-100, and 10% normal rabbit serum, and subsequently incubated overnight at 4°C with primary antibodies (Table 1). On the next day, after washing in PBS, cells were incubated for 1 hour at room temperature with one of the following secondary antibodies (Table 1). Finally, after rinsing, observed by fluorescence microscopy (BX41; Olympus Corp., Tokyo, Japan) equipped with a digital camera (DP70; Olympus Corp.).

Histology

For light microscopy, fixed BMSCs-RPE cells embedded in paraffin. We cut 5-µm thick sections. For descriptive histology and quantitative analysis, sections were stained with hematoxylin and eosin staining. Briefly, the sections were Deparaffinized, after which hydration was performed with descending degrees of alcohol 96% for 5 min in hematoxylin dye for 10 min. In order to eliminate extra dies, they were once placed inside alcohol acid container and to fixate the color, it was immersed in lithium carbonate, then washed with water three times, and then placed in eosin dye for 3 min. Eventually, it was placed inside alcohol containers 96 and 100%, and made transparent in two xylol containers. All observations and photography were performed using a fluorescent microscope (BX41; Olympus Corp., Tokyo, Japan) equipped with a digital camera (DP70; Olympus Corp.).

Electron microscopy

For transmission electron investigation, BMSCs-RPE cells were washed with PBS, and fixed in 1 ml of glutaraldehyde solution 2.5%

at room temperature for one hour and half. The samples were washed three times by phosphate buffer 0.1 M each time for half an hour at 4°C so that the non-reacted and free glutaraldehyde would be removed from the samples. Thereafter, the samples were placed inside agar. Once washed, tetroxide osmium 1% was added to the sample and then kept for one hour. Next, the sample was transferred to pure resin for 24 h. After molding using ultramicrotome and using a glass knife, 500 nm cut resin molds were prepared. After drying and attaching the cuts onto the slide, the sample were staining with uranyl acetate and lead citrate. After washing and observing under the optical microscope in case the sample was suitable, a slide was attached using Entellan™ adhesive and then imaged. Thereafter, the samples were stained using uranyl acetate and lead citrate, whereby imaging was done using transmission electron microscope.

To perform scanning electron microscopy, BMSCs-RPE cells were washed twice with PBS and fixed with 2.5% glutaraldehyde (Sigma Aldrich) for 24 hours and then were dried and washed twice using serial ethanol concentrations (25, 45, 55, 65, 75, 85, 90, 95 and 100%). The samples were placed at room temperature overnight and dried, mounted on an aluminum substrate and covered with a gold dispenser. The images were obtained by electron microscopy.

Melanin granules in BMSCs-RPE cells

Image J software (ImageJ 1.46r, Wayne Rasband, National Institutes of Health, USA, http://imagej.nih.gov/ij.) was used to evaluate the concentration of melanin granules in the cytoplasm of differentiated and normal cells. To do end, the procedure was performed on images with 100x and 200x magnification. First, adjust color thresholds were performed for the images, and black and white thresholds were used after specifying the melanin pigments. The size of melanin particles was determined and their number was calculated and counted in the analysis section. The data were reported as the total number of calculated granules, the total area occupied by melanin and the percentage of space occupied by melanin. The mean number of melanin granules was also analyzed after three replications.

Statistical analysis

Data were obtained and expressed in mean ± SEM values. The collected data were analyzed using one-way analysis of variance (RM one-way ANOVA) as well as Tukey's test in GraphPad Prism ver. 8. P-value<0.05 was considered as the significance level in all tests. Paired two tailed t-test was also used to analyze the data between the two groups.

Results

Cell culture

Rat bone marrow-derived mesenchymal stem cells were used in the present study. The results of cell culture showed that the cells had a spindle-shaped appearance and adhered to the flask bottom. The results of the colony forming unit (CFU) showed that these have the ability to form a colony (Figure 1 top panel). Immunocytochemistry showed the number of positive cells compared to the total number of cells. The results showed that CD90, CD166, and fibronectin were expressed by 95.33%, 96.26% and 100% of the cells. These results confirmed that the cultured cells are mesenchymal stem cells.

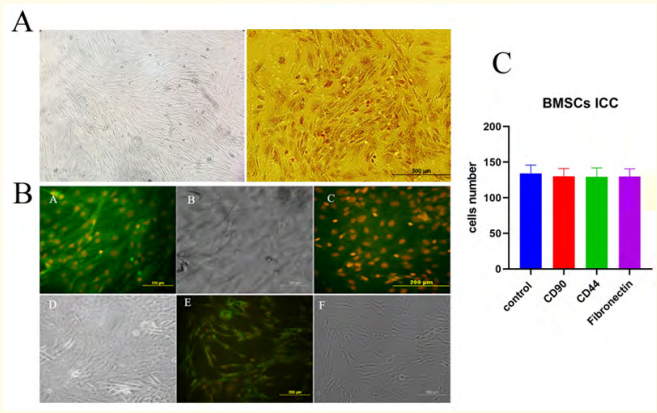

Figure 1: A, bone marrow mesenchymal stem cells cultured in the third passage shows colony forming unite. B, Immunocytochemistry of rat bone marrow mesenchymal stem cells after the third passage. A and B represent the CD90 marker, C and D represent the CD166 marker, and E and F represent the fibronectin marker. C, diagram shows the expression of mesenchymal cell markers by bone marrow mesenchymal stem cells. Magnification: 200 µm top panel for A, B, 200 µm bottom panel for A-F. Error bars, SD for three repetitions in each case (* P < 0.05, ** P < 0.01, *** P < 0.001, *** P < 0.0001).

The results of native rat RPE culture (Figure 2) showed that RPE gradually lose their cytoplasmic pigment after being isolated and attached to the flask bottom as compared with mesenchymal stem cells cultured in passage zero (top panel). The results showed that these cells gradually lose their melanin pigment seven days after culture and enter the cell division phase (top panel C, D). Also, the morphology of these cells is polygonal and epithelial at first, but gradually changes and they are even seen in a spindle shape in the early stages of culture. The results of immunocytochemistry (Figure 2 bottom panel) showed that 76.52% of the cultured cells express the RPE65 marker (A, B) and 62.06% of the cells show the ZO-1 marker (C, D).

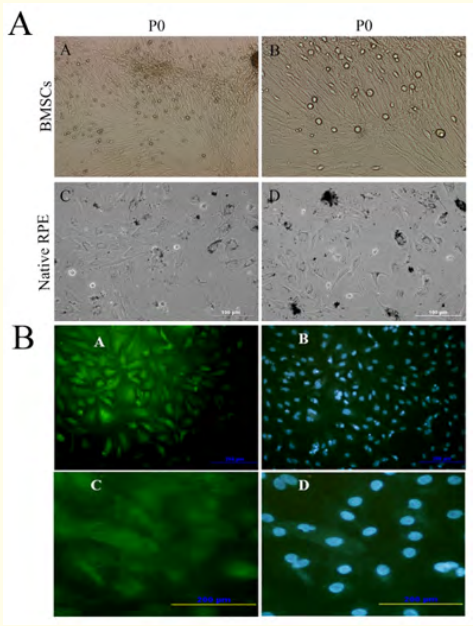

Figure 2: Top panel (A), represent bone marrow mesenchymal stem cells (A, B) compared to the native RPE cells (C, D). Bottom panel (B), show immunocytochemistry of native RPE cells. In bottom panel, A and B show the RPE65 marker and C and D show the ZO-1 marker. The nuclei of the cells are labeled with DAPI. Magnification: Top panel 200 µm for A, B and 100 µm for C, D and bottom panel 100 µm for A, B and 200 µm for C, D.

In this study, we investigated the differentiation of BMSCs into RPE over the course of 60 days. Figure 3 shows the schematic diagram of the process of differentiation and investigation of RPE.

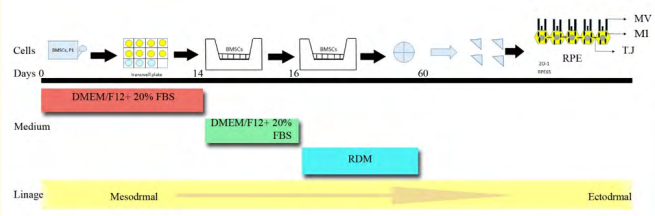

Figure 3: Schematic diagram of the process of differentiation and study of pigmented cells derived from bone marrow stem cells.

Morphological investigation (Figure 4) showed a change to non-spindle shaped morphology in BMSCs-RPE cells and also the appearance of melanin pigment in the cytoplasm over the course of 60 days indicate that these cells have been differentiated to RPE.

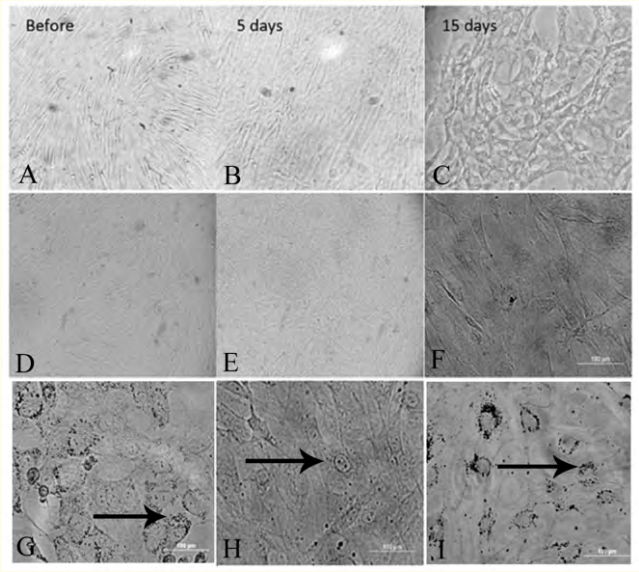

Figure 4: Images showing the differentiation of BMSCs on days 5 to 60 (B-H). The appearance of the cells and their pigmentation are noticeable in the images shown with the arrow. Cytoplasmic pigments of melanin (arrows G, H) are seen in BMSCs-RPE compared to native RPE cells (arrow I) at the end of cell differentiation. Magnification: 50 µm for A, B, D, F and 100 µm for C, F, G-I.

Immunocytochemistry was used to confirm the BMSCs-RPE cells (Figure 5). The results showed that 85.82% and 33.22% of the cells expressed RPE65 (D, E) and ZO-1 (F) (Figure B) compared to the control group (A, B) and total number of counted cells.

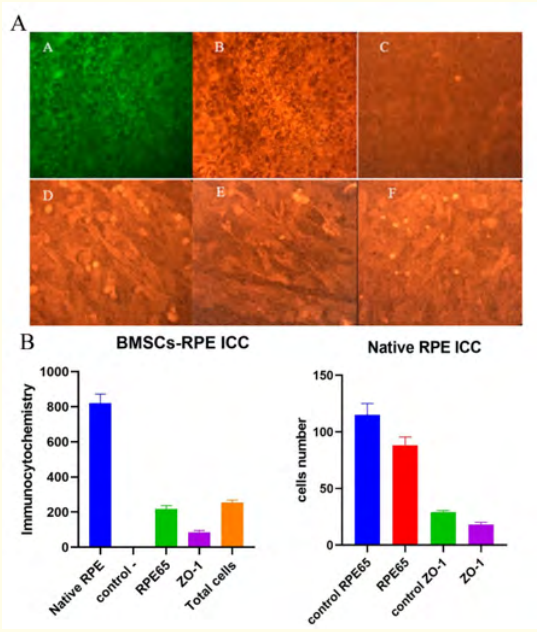

Figure 5: The top panel (A) shows the immunocytochemistry of pigmented cells. A, B represent RPE65 marker of native RPE cells (positive control), C negative control. D, E represent the RPE65 marker and F represents the ZO-1 marker. Magnification: 50 µm for A-C and 100 µm for D-F. The bottom panel (B) shows the expression of specific markers of BMSCs-RPE compared to native RPE cells. Error bars, SD repeated three times in each case (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001).

The data of melanin investigation (Figure 6) showed a statistically significant difference between day one, days 30 and 60 after differentiation (P < 0.0006 and p < 0.0011). There was also a statistically significant difference between day 30 and day 60 after differentiation (P < 0.0123). There was also a statistically significant difference between day 30 and seven-day native RPE cells (P < 0.0426). However, there was no statistically significant difference between day 60 and native seven-day normal RPE cells (P = 0.7142) (Figure 6 and Table 2). The results of volumetric and three-dimensional study of melanin pigment dispersion in the cytoplasm of BMSCs-RPE showed that melanin was absent on day 1 and was concentrated at the apex of BMSCs-RPE cells on days 30 and 60 compared to native RPE cells.

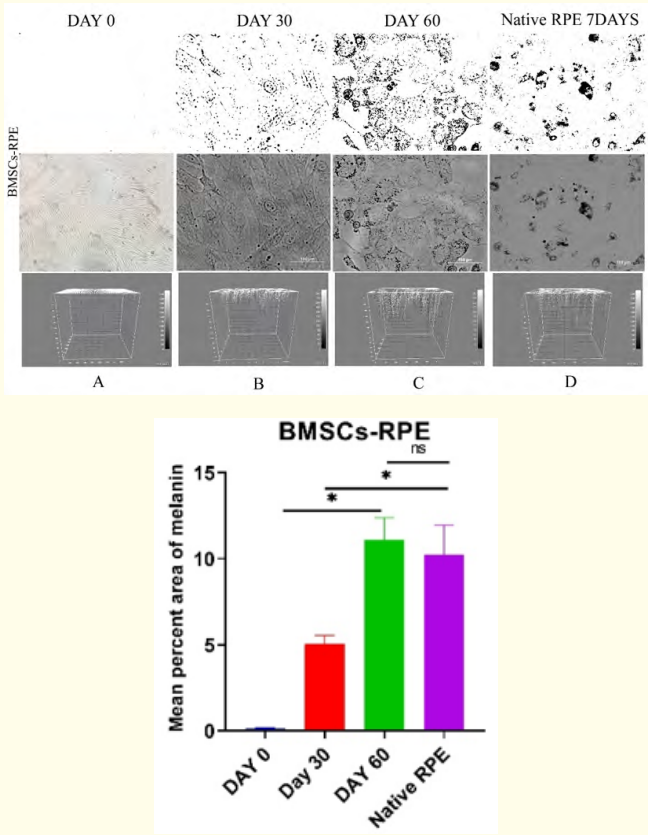

Figure 6: Represent melanin concentration in BMSCs-RPE cells. The top panel showed the increasing in number of melanin granules within the cytoplasm of BMSCs-RPE cells compared to mesenchymal stem cells (A) and native RPE cells (D) in culture medium. The bottom panel is a volumetric and three-dimensional image of how melanin is distributed in BMSCs-RPE cells compared to native RPE cells. E, represents the average percentage of melanin pigment in BMSCs-RPE cells over the course of 60 days compared to native RPE. Magnification: 100 µm for day 30, 60 and 50 µm for day zero. Error bars, SD repeated triple in each case (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001).

Histology and electron microscope

The results of hematoxylin and eosin staining for apical microvilli of BMSCs-RPE cells cultured on the filter over the 60 days showed that they have epithelial appearance with a round central nucleus (Figure 7 A, B arrows) and microvilli-like structures in the apical side of these cells (Figure 7 C, D arrows).

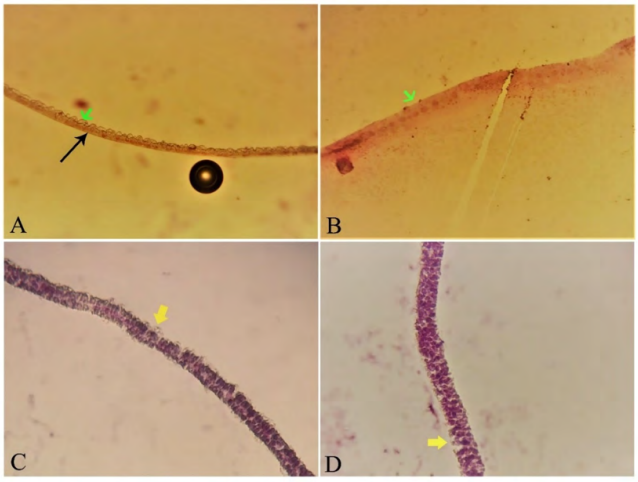

Figure 7: Morphology and apical microvilli of BMSCs-RPE cells stained by hematoxylin and eosin. The epithelial morphology of the cells and round is shown in A and B. Apical microvilli are also seen in C and D. Magnification: 50 µm for A, B and 100 µm for C, D.

The results of scanning electron microscope (Figure 8 top panel) showed that apical microvilli were seen on the outer surface of the BMSCs-RPE. Native RPE have two types of microvilli at their apex where they phagocyte outer segment of photoreceptors. It seems that BMSCs-RPE cells have a number of apical microvilli (top panel D arrowhead). The results also showed a cell junction formed between the differentiated cells (top panel C arrow).

The results of transmission electron microscopes of BMSCs-RPE cells (Figure 8 bottom panel D-F) compared to native RPE (A-C) showed cell junction between native RPE cells (C arrow) and between BMSCs- RPE (D, E arrowheads). The cytoplasm of BMSCs-RPE cells also contains the numerous pigment melanin (F arrow).

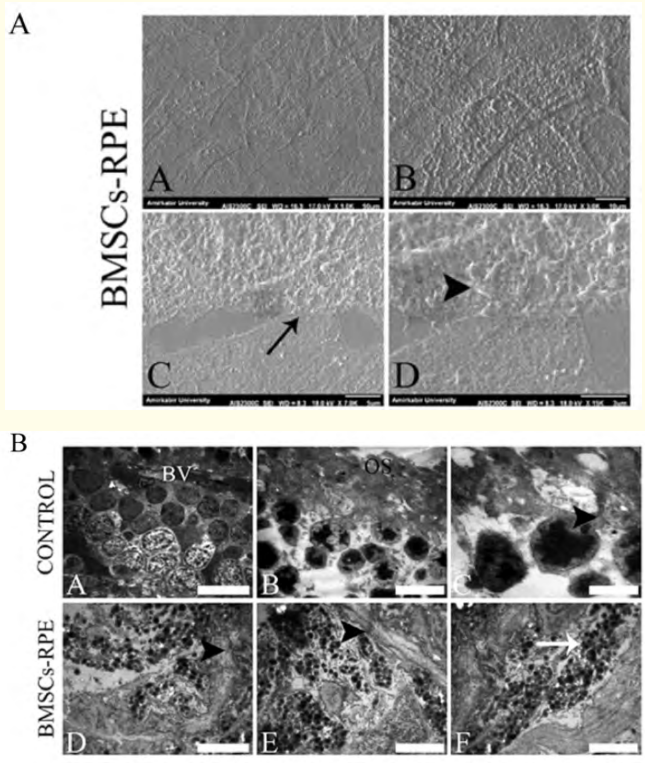

Figure 8: Represent Electron microscope of BMSCs-RPE cells. Top panel (A) demonstrated cell junction between cells as well as apical microvilli by arrow (D) and arrowhead (C). Bottom panel (B) transmission electron microscope images of BMSCs-RPE cells. A-C show native RPE cells and D-F show BMSCs-RPE. The arrowheads in C, D, and E show the cell connection between the cells, and the arrow in F shows the melanin. Abbreviations; BV: Blood vessels, OS: outer segment of photoreceptors. Magnification: top panel 3, 5, 10 and 50 µm respectively.

Discussion

The present study investigated the differentiation of rat bone marrow mesenchymal stem cells (BMSCs) into RPE as well as their cellular characteristics. Results showed that BMSCs can differentiate into RPE and express specific proteins RPE65 and ZO-1. Results also showed that differentiated cells, compared to normal cells, have apical microvilli and cytoplasmic pigment melanin, and that there is a cell junction between them.

The goal of cell therapy for RPE cells in AMD is to replace old and defective cells with new and young cells. Since iPSCs cells that undergo an aging process, it is no appropriate to use them at this moment. In the present study, RPE cells were seen in culture medium after 6-8 weeks and have melanin pigment, but their appearance is not completely polygonal. Cell culture studies show that iPSCs and ESCs can differentiate into RPE [26,27]. These studies have shown that RPE cells are obtained spontaneously by removing bFGF from the culture medium, and become pigmented and acquire a polygonal morphology when become condensed after 20-30 days.

The induction method by small molecules was used in the present study. In a similar study, Duarri., *et al*. also used the induction method to differentiate ocular-derived cells to produce RPE [28]. We also obtained same results regarding the RPE production from human adipose tissue-derived stem cells (hADSCs) that could be treated and replaced in retinal degeneration [25]. A mixture of substances and hormones was used in the present study to perform the differentiation process and deliver it to the cell in the form of small molecules to produce RPE cells by induction method. Studies show several differentiation media for different cell types, including human and mouse, and there are differences between their methods even in the case of human cells. But the important point is the use of substances that can create and maintain cell junction and barrier properties in the produced RPE [29]. In general, the differentiation media used to produce RPE include DMEM, SFM, MEM, DMEM/F12, DMEM low glucose, DMEM high glucose [30]. Studies show that the type of basal medium affects the cell behavior and morphology [31] in the culture medium so that the use of MEM medium creates cells in the culture medium that have a lot of cytoplasmic melanin content and there are microvilli on their apical surface. However, the use of DMEM/F12 medium causes the formation of cells that have the fibroblast-like appearance with a low melanin content as well as apical microvilli [32]. The present study revealed that BMSCs gradually became pigmented over time after being exposed to RPE differentiation medium. Dark granules were observed in their cytoplasm after 60 days, while the basal medium of these cells was DMEM/F12.

The RPE plays an important role in the health of the retina. Changes in the RPE layer are seen in many degenerative retinal diseases, including AMD. One of the unique characteristics of RPE is their cytoplasmic granules. These granules begin to form during the embryonic stage and accumulate in the cytoplasm after birth due to several factors, including cell damage and aging, and one of the main diagnostic symptoms of AMD disease is the accumulation of cytoplasmic pigments. Overall, RPE have four types of granules, which include: lipofuscin, melanolipofucin, melanosome and mitochondria [33-35]. In humans, melanin granules are usually observed between days 27-30 of the embryonic period. They usually represent all maturation stages in 14 embryonic weeks [36]. The appearance of granules such as melanin in RPE cells indicates that these cells have entered the stage of development and polarity. Previous studies have reported melanin production in RPE seven days after differentiation (first to fourth stages of melanin maturation) and the presence of granules at the final stage of melanin maturation fifteen days after RPE differentiation [30]. However, the present study showed melanin granules in BMSCs-RPE cells after 60 days, which is consistent with results of other studies regarding other stem cells such as iPSCs [28,36,37] and ESCs [36,38] and even mesenchymal stem cells [22,24,25,36]. Studies show that extracellular matrix can play a role in the melanin reconstruction in primary RPE cells. These matrices include corneal endothelial matrix, laminin with fibroblast growth factor, cell culture inserts and amniotic membrane [36]. A cell culture insert matrix (Transwell plate) was used in the present study and it is supposed that more cellular characteristics like melanin, apical microvilli and cell junction are related to it. Also, we investigated cytoplasmic dispersion of melanin at the apex of the RPE cell. In this regard, Bermond., *et al*. [39] showed that melanolipofsin is located in the apex to middle part, and lipofuscin in the basal-lateral and basal parts. There is also a small melanin content in the apex and a fourth phenotype of melanolipofuscin in the apex.

In the present study, apical microvilli were observed in differentiated RPE 60 days after induction. Studies show that apical microvilli are observed on days 7 to 15 after direct cell differentiation [30] or after co-culture with swine RPE [22], which is consistent with studies showing that apical microvilli are found in RPE that are differentiated from iPSCs, ESCs and MSCs.

Cell junctions modulates basal-apical polarity, cell proliferation, and cell survival [40,41]. RPE play an important role in the formation of retinal blood barriers due to their tight junction [29]. However, there is no intercellular junction at the time of *in vitro* cell division. On the other hand, these cells have microvilli on

their apical surface and lose their polarity during cell division and, therefore, can enter mitosis phase. But they acquire their polarity and cell junctions during maturation. It is possible to study such changes using an electron microscope. Therefore, we were able to show a cell junction between the generated BMSCs-RPE cells as well as the melanin granules using a scanning and transmission electron microscope. These findings are consistent with studies that show the presence of microvilli at the apex of iPSCs-derived RPE [28]. In general, the produced BMSCs-RPE in our study seem to show some characteristics of RPE. However, cell junction and trans epithelial resistance in these cells as well as expression of specific genes and proteins secreted from the apical and basal parts of these cells were not investigated in the present study and may indicate the need for further studies.

Conclusion

Bone marrow mesenchymal stem cells can be considered a good cell source in retinal diseases including AMD considering their immunological and neuroprotective properties and ability to differentiate into different cell lines. BMSCs-derived RPE exhibit the characteristics of RPE such as apical microvilli, melanin, and cell junction. These cells can be used as a promising cellular source in cell-based therapies for retinal diseases.

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' Contributions

- Zahra Hajiha contributed to the study material preparation, data collection and write the primary draft.
- Hamid Aboutaleb Kadkhodaeian contributed to the study conception and design, material preparation, data collection, analysis and write the manuscript.
- Hamidreza Sameni contributed to the study material preparation.
- Mona Farhadi contributed to the study material preparation.
- Sam Zarbakhsh contributed to the study material preparation.
- All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available.

Ethics Approval

Eyes samples were collected and carried out according to (ethical standards of the responsible committee on Animal experimentation) (institutional and national) and with Helsinki Declaration of 1975, as revised in 2008.

Conflict of Interest

Authors have no conflict of interest.

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