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In vitro induction and intraocular application in oxygen-induced retinopathy of adipose-derived mesenchymal stem cells

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Purpose: We designed a study to find theoretical evidence for the induction, movement, fusion, proliferation, and safety of human adipose mesenchymal stem cells (hADSCs) in intraocular application.
Methods: HADSCs were induced to confirm that they can express the characteristics of endothelial cells (ECs) in vitro. HADSCs were intraocularly injected into oxygen-induced retinopathy (OIR) mice to check the movement, fusion, proliferation, and prognosis in vivo. Electron microscopy was used to check retinal changes to confirm the safety of hADSCs in intraocular application.
Results: After induction, hADSCs expressed von Willebrand Factor (vWF), the cell marker of ECs. The hADSCs were distributed above the retina after an intravitreal injection in the OIR mice. The injected cells did not fuse with the retina and gathered in the central and peripheral areas, which is the lesion area of the OIR model. Five days after the hADSC intravitreal injection, the area of neovascularization was reduced by 94.83% compared with that of the OIR group. Hematologic staining and electron microscopy did not show noticeable proliferation and degeneration of the retina.
Conclusions: This study provides evidence for the intraocular application of hADSCs.

Retinal vascular diseases, such as diabetic retinopathy (DR), can cause irreversible vision loss. With the population's increase in age and lifestyle changes, the incidence of these diseases is increasing every year [1-3]. The main pathological change in DR is damage to retinal endothelial cells (ECs), which leads to retinal neovascularization [4]. The current treatments for these diseases, including antivascular endothelial growth factor (VEGF) therapy, are not satisfactory, as they do not fundamentally prevent the progression of the diseases [5,6]. Maintaining or restoring the function of RPECs or ECs is essential.

Mesenchymal stem cell (MSC) therapy, due to its unique advantages, such as cell replacement, environmental regeneration, paracrine, and no immune rejection [7,8], brings hope for intractable retinal diseases and has been proven to have positive results [9,10]. Many studies have confirmed that MSCs could promote angiogenesis [11,12]. However, one study showed that human Wharton's jelly-derived MSCs were differentiated to express human leukocyte antigen DR (HLA-DR) after intravitreal transplants, which can lead to severe retinal inflammation [13]. Moreover, another report showed that one patient developed fibrosis proliferation after MSC intravitreal transplantation [14]. The differentiation, proliferation, prognosis, and safety of MSCs for in vivo therapy remain controversial. However, among the different tissue sources of MSCs, adipose mesenchymal stem cells (ADMSCs) and bone marrow-derived stem cells (BMSCs) are mainly studied, and they exhibit similar surface molecular markers and differentiation abilities [15]. ADMSCs are easier to obtain, have higher proliferation rates than BMSCs, and thus have more extensive prospects [16,17]. Therefore, in this study, we assessed the ability of human adipose mesenchymal stem cells (hADSCs) to express the characteristics of ECs after induction in vitro to evaluate the theoretical feasibility of maintaining or restoring the function of ECs after intraocular application. Then, hADSCs were intraocularly injected into a pathological model (oxygen-induced retinopathy [OIR] model) to check the movement trajectory, fusion, proliferation, and prognosis of the hADSCs in the intraocular application using morphological methods. Finally, electron microscopy and hematoxylin and eosin (H&E) staining were used to assess retina changes to confirm the safety of intraocular use of hADSCs. The findings provide theoretical support for the intraocular application of hADSCs.

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METHODS

Culture, characterization, and labeling of hADSCs: The second passage of hADSCs was obtained from the Tissue Engineering Center of Peking Union Medical College, China.

The hADSCs were recovered in 37 °C warm water, added to the culture medium, and cultured in a humidified incubator. Then, 48 h later, one-half of the culture medium was replaced, and the cells were subcultured every 3–4 days or at 80% confluence. The third passage of hADSCs was used for subsequent experiments. The culture medium included DMEM/F12 (Gibco, Grand Island, NY), 10% fetal bovine serum (Gibco), 1% penicillin/streptomycin (Gibco), and 0.2 mM L-ascorbic acid-2-phosphate (Sigma, Burlington, MA).

Characterization of the hADSCs was performed with flow cytometry on the specific surface antigens CD29, CD34, CD44, CD105, Flk-1, and HLA-DR (BD Biosciences, Franklin Lakes, NJ) according to the manufacturer's recommendations,. Data were analyzed using the analysis software FlowJo V10 (FlowJo, Ashland, OR).

The hADSCs were labeled with CM-Dil according to the manufacturer's recommendations (Thermo Fisher Scientific Inc., Waltham, MA). The cells were digested with 0.25% trypsin, centrifuged, and washed with a serum-free medium. The supernatant was discarded, and 4 µM of CM-DiI was added. Then, the cells were incubated at 37 °C for 30 min. Subsequently, the cells were washed with phosphate-buffered saline (PBS; 1X; 137 mM NaCl, 2.7 mM KCl, 10 mM $NaPO_4$, 2 mM KPO_4, pH 7.4) three times (5 min each time), the culture medium was added, and the cells were observed under a microscope after adherence.

Induction and identification of hADSCs: The hADSCs were seeded in a six-well plate at a density of 1×10^4 cells/well and adhered to the plate for 12 h. Then, the experimental group cells were treated with 100 mg/l penicillin, 50 ng/ml VEGF (R&D Systems, Minneapolis, MN), and 20 ng/ml basic fibroblast growth factor (bFGF; R&D Systems). Control group cells were treated with the same amount of PBS. Cells were cultured in a humidified incubator, and half of the culture medium was changed in 2 days. After induction for 14 days, the cells were observed under a microscope. Then, as described above, after washing and fixing, 2 µg/ml von Willebrand Factor (vWF) monoclonal antibody (Thermo Fisher Scientific Inc.) was added, and the cells were incubated at room temperature for 60 min. About 50 µl fluorescein isothiocyanate (FITC)-labeled goat anti-mouse secondary antibody was added. Then, the cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) and observed using a fluorescent microscope.

OIR model: Pregnant C57BL/6J mice were provided by the Laboratory Animal Center of Southern Medical University, China. All animal experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local animal welfare committee. The OIR model was created as described by Smith [18] (the OIR group). Seven-day-old (P7) mice pups and their mothers were exposed to 75±2.0% oxygen for 5 days (P7 to P12). Then, the P12 mice were transferred to room air until P17. Half of the P12 OIR group mice were intravitreally injected with CM-Dil-labeled hADSCs (the injected group). Mice pups were kept in a normal environment (the control group). The nursing mothers were rotated from high oxygen to room air every 24 h to prevent oxygen toxicity.

Intravitreal injection: Mice were anesthetized with an intraperitoneal (IP) injection of 10% chloral hydrate (2.5 ml/kg). The eyelid was separated under a microscope, and the eyes were dilated with one drop of 0.5% tropicamide. The eyeball was exposed, and after paracentesis of the anterior chamber to release part of the aqueous humor, 1 µl of CM-Dil-labeled hADSCs (at a concentration of 1×10^5/ml) was injected into the vitreous cavity using a 33-G Hamilton syringe (Hamilton Company, Reno, NV). The needle was inserted into the vitreous cavity in the direction of the optic nerve about 1 mm behind the corneoscleral limbus and was taken out after 30 s. Erythromycin ointment was applied, and then the eyelids were closed. Intravitreal injection in the hADSC injection group was performed at P12.

Histological evaluation: After euthanasia, the enucleated eyes were fixed with 4% neutral paraformaldehyde for 24 h, dehydrated by gradient ethanol and transparent xylene, and embedded in paraffin wax. The retina was continuously sliced with a thickness of 4 µm parallel to the sagittal axis of the optic nerve. The slices were baked overnight, deparaffinized with xylene, hydrated with gradient ethanol, stained with hematoxylin and eosin, and observed under a microscope (Zeiss Axioplan 2 Imaging, Zeiss, Gottingen, Germany). One slice was randomly selected from every five slices. Histological evaluation of the retinas from the control, OIR, and HADSC injection groups was performed at P17.

Retinal flat mount: Mice pups were anesthetized and received a retro-orbital injection of fluorescein isothiocyanate dextran, described by Li et al. [19]. Then, 10 s later, the mice were euthanized using pentobarbital (150 mg/kg, intraperitoneal injection). Enucleated eyes were fixed in 4% paraformaldehyde for 30 min at room temperature and then washed three times in PBS. The retinas were separated from the sclera, RPE, lens, and cornea, and cut into four parts. Water-soluble mounting tablets were used for mounting, and coverslips were added. The retinal flat mounts were photographed at 5X original magnification with fluorescence microscopy (Zeiss Axioplan 2 Imaging). The combined exposure time of the retinal flat mounts was 1.5 s. The retinal segments

were merged to generate an image of the total retina (Photoshop 2020; Adobe Systems Inc., San Jose, CA). Flatmounted retinas from the control, OIR, and hADSC injection groups were examined at P17. Retinal neovascularization and the total retina were measured by outlining the corresponding areas and analyzed using Image Pro-Plus 5.0 software (Media Cybernetics Company, Silver Spring, MD).

Transmission electron microscopy: After euthanasia, the enucleated eyes were fixed with 2.5% glutaraldehyde at 4 °C overnight, 1% osmium tetroxide at room temperature, and ethanol. After incubation in acetone for 20 min, the eyes were treated with 50% (1 h), 75% (3 h), and 100% (overnight) epoxy resin and heated at 70 °C overnight. The eyes were sliced into sections of about 70 nm. The sections were stained with 3% uranyl acetate and 3% lead citrate for 15 min at room temperature and observed under an electron microscope. One slice was randomly selected from every five slices. Retinal ganglion cells (RGCs) and cells of the outer nuclear layer from the control, OIR, and HADSC injection groups were examined at P17.

Statistical analysis: Data have been reported as the mean ± standard deviation of the mean (SDM). Statistical analysis was performed using statistical software (SAS Institute Inc., Cary, NC). Data were analyzed using independent samples *t* tests to compare the two groups' differences. A p value of less than 0.05 was considered statistically significant.

RESULTS

Culture, characterization, and labeling of hADSCs: In the second passage, hADSCs grew well and had spindle and polygonal shapes (Figure 1A). The flow cytometry results showed that the hADSCs expressed low levels of the hematopoietic and endothelial markers CD34 (0.8%) and HLA-DR (0.5%), and high levels of CD29 (95.3%), CD44 (99.8%), CD105 (99.7%), and Flk-1(99.0%). After CM-Dil labeling, the membrane of the hADSCs showed red fluorescence, and the nucleus showed no fluorescence (Figure 1B).

Induction of hADSCs: After being inducted into endothelial-like cells, the corners of the hADSCs had round shapes (Figure 1C). Expression of vWF, an endothelial cell marker, was absent (no fluorescence) in the control group (Figure 1E). However, expression of vWF was present (green fluorescence) in the experimental group (Figure 1H), and the positive rate was 100%. The difference in the positive rate between the two groups was statistically significant ($p<0.001$). The nuclei stained with DAPI showed blue fluorescence in the two groups.

Intraocular tracking of hADSCs: The histological staining of the P17 mice in the control group showed no neovascularization breaking through the retina's inner limiting membrane (ILM; Figure 2A). The P17 mice in the OIR group showed extensive neovascularization that broke through the ILM (Figure 2B). The P17 mice in the hADSC injection group showed no apparent neovascularization breaking through the ILM, and the injected cells were above the ILM and were not fused with the retina (Figure 2C,D).

The retina flat mount of the P17 mice in the control group showed that the retina blood vessels were smooth, without neovascularization, and had no perfusion area (Figure 2E). The P17 mice in the OIR group showed extensive highly green fluorescent neovascularization in the periphery area and non-perfusion in the central area of the retina (Figure 2F). The P17 mice in the hADSC injection group showed an area of neovascularization and non-perfusion that was significantly reduced compared with that in the OIR group. Many labeled hADSCs with red fluorescence were noted above the neovascularization and non-perfusion area of the retina (Figure 2G,H).

Quantification of retinal neovascularization: The retinal neovascularization areas of the normal group, OIR group, and MSC group were 0, 81,205±9217 μM^2, and 4201±623 μM^2, respectively. Compared with the normal group, the neovascularization area of the OIR group was significantly increased ($p<0.0001$), and the neovascularization area of the MSC group was significantly lower than that of the OIR group ($p<0.0001$; Figure 3).

Proliferation, degeneration, and changes after injection of hADSCs: The histological staining results for the hADSC injection group at P17 (Figure 2C) showed that the structure of each layer of the retina was clear, and the number of retinal cells was not significantly different from that in the control group at P17 (Figure 2A). There was no noticeable proliferation in the hADSC injection group at P17. Electron microscopy of the control groups showed that the RGCs and the cells of the outer nuclear layer had a defined plasma membrane and uniformly distributed chromatin (Figure 4A,D). There were fewer autophagosomes in the hADSC injection group (Figure 4C,F) than in the OIR group (Figure 4B,E) and the control group. There was no noticeable difference between the normal group and the MSC group.

DISCUSSION

Stem cell therapy brings new hope for the treatment of retinal diseases [20,21], but "stem cell therapies" have not been approved by the Food and Drug Administration (FDA) and are still controversial for clinical application. This study was

designed to find theoretical evidence for the induction, prognosis, proliferation, and safety of hADSCs for intraocular application. We observed the feasibility of induced hADSCs expressing EC-like characteristics. Furthermore, we used HE, retinal flat assessment, and electron microscopy to track and evaluate the movement trajectory, fusion, proliferation, and safety of hADSCs in intraocular application in vivo. The study results provide theoretical support for the intraocular use of hADSCs.

This study showed the feasibility of hADSCs expressing RPEC-like and EC-like characteristics in a specific environment. This result suggests the possibility of hADSCs maintaining or restoring the function of RPECs or ECs in AMD or DR. The disease usually coexists with multiple etiologies, such as inflammatory factors also accompanying DR. We used hADSCs in this study, not induced cells, because we wanted to avoid limiting the effects of primitive cells.

Retinal flat mounts were used to examine the distribution and movement of ADMSCs. The results showed that the ADMSCs were distributed above the retina and gathered in the central non-perfusion and peripheral neovascular area, which is the lesion area in the OIR model. This means that

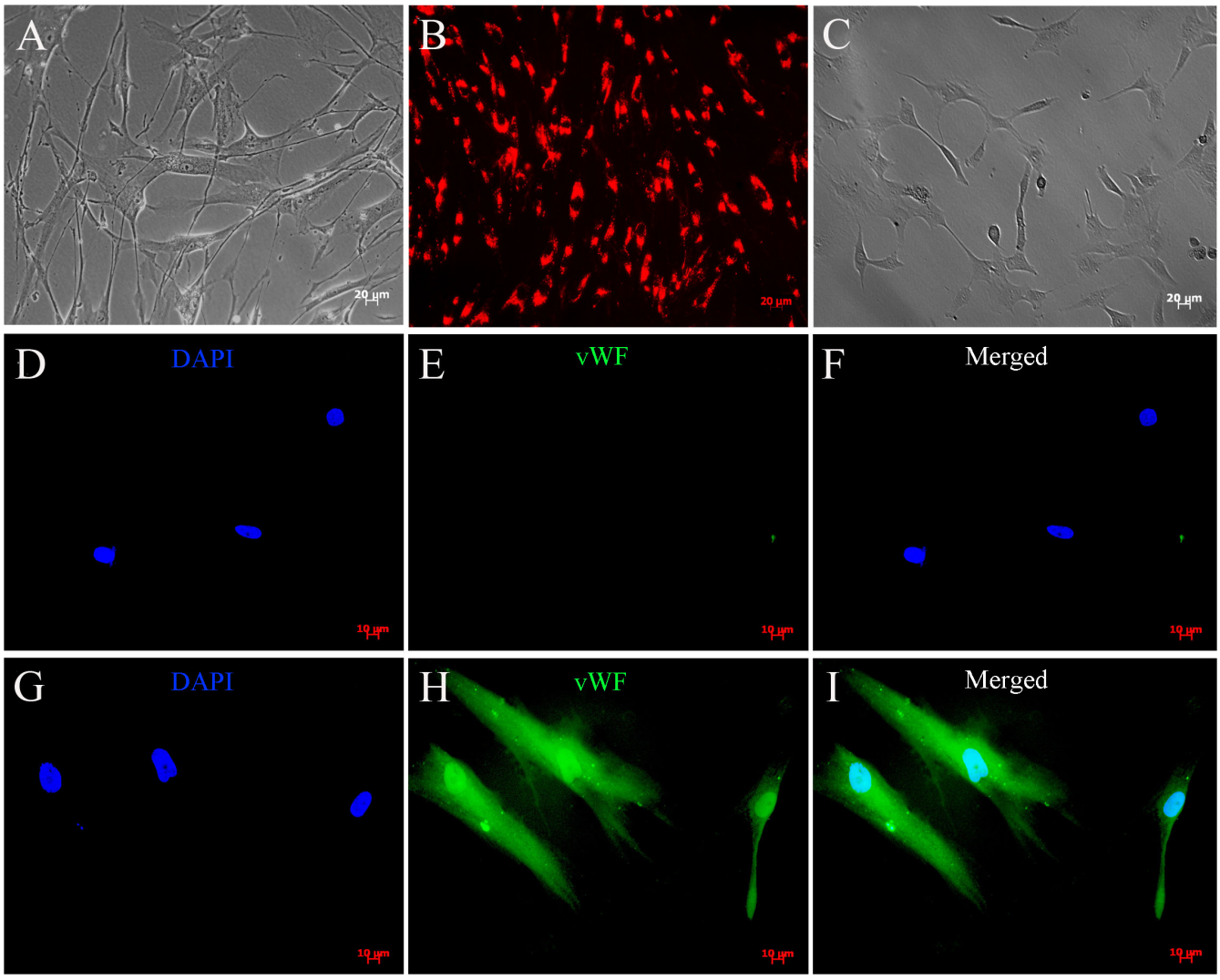

Figure 1. Culture, labeling, and immunofluorescence staining of hADSCs. **A**: In the second passage, the human adipose mesenchymal stem cells (hADSCs) show spindle and polygonal shapes. **B**: The membrane of the hADSCs shows red fluorescence, and the nucleus shows no fluorescence. **C**: After induction, the corners of the hADSCs appear rounded. **D**, **E**, **F**: The expression of von Willebrand Factor (vWF) is absent (no fluorescence) in the control group. **G**, **H**, **I**: The expression of vWF is present (green fluorescence) in the experimental group. The nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI) shows blue fluorescence in each group. Scale bar = 20 μm, 200X; scale bar = 10 μm, 400X.

ADMSCs may automatically gather in the diseased area. These results are consistent with those of Wang et al. [22]. Those authors used human umbilical cord mesenchymal stem cells (hUCMSCs) and injected them into an elevated intraocular pressure (IOP) model. They confirmed that 7 days after transplantation, a small fraction of cells were seen on the ILM of the retina, suggesting that hUCMSCs can migrate to the site of retinal injury. Moreover, another study confirmed that mouse BMSCs can migrate to the limbal stroma and wound healing edge after subconjunctival injections [23]. However, Ghazaryan et al. showed no migration of cells after applying MSCs to corneal neovascularization [24]. The migration results seem to differ from those for different MSCs under other pathological conditions. The present results showed the automatic migration of hADSCs after intravitreal injection in OIR mice, and it is worth understanding the mechanism of automated migration in future studies.

The fusion of cells with the host is a fundamental issue that directly affects prognosis, side effects, and future use. The present results showed that hADSCs were distributed in the vitreous cavity and did not fuse with the retina in an environment of retinal neovascularization caused by ischemia and hypoxia. Similar to this study, around 12 weeks after MSC intraocular injection, Ezquerra et al. detected that most donor cells remained in the vitreous cavity and did not integrate into the retina [25].

However, other studies showed that intravitreally injected BMSCs were fused with rat retinas in different models [26,27]. We thought that the fusion after intravitreal injection might depend on the cell's molecular weight, the ILM [28], glial endfeet [29], and different microenvironments [30]. It has been proposed that the therapeutic mechanism of MSCs is mainly through the secretion of trophic factors [31], and that the exosomes are paracrine effectors of MSCs

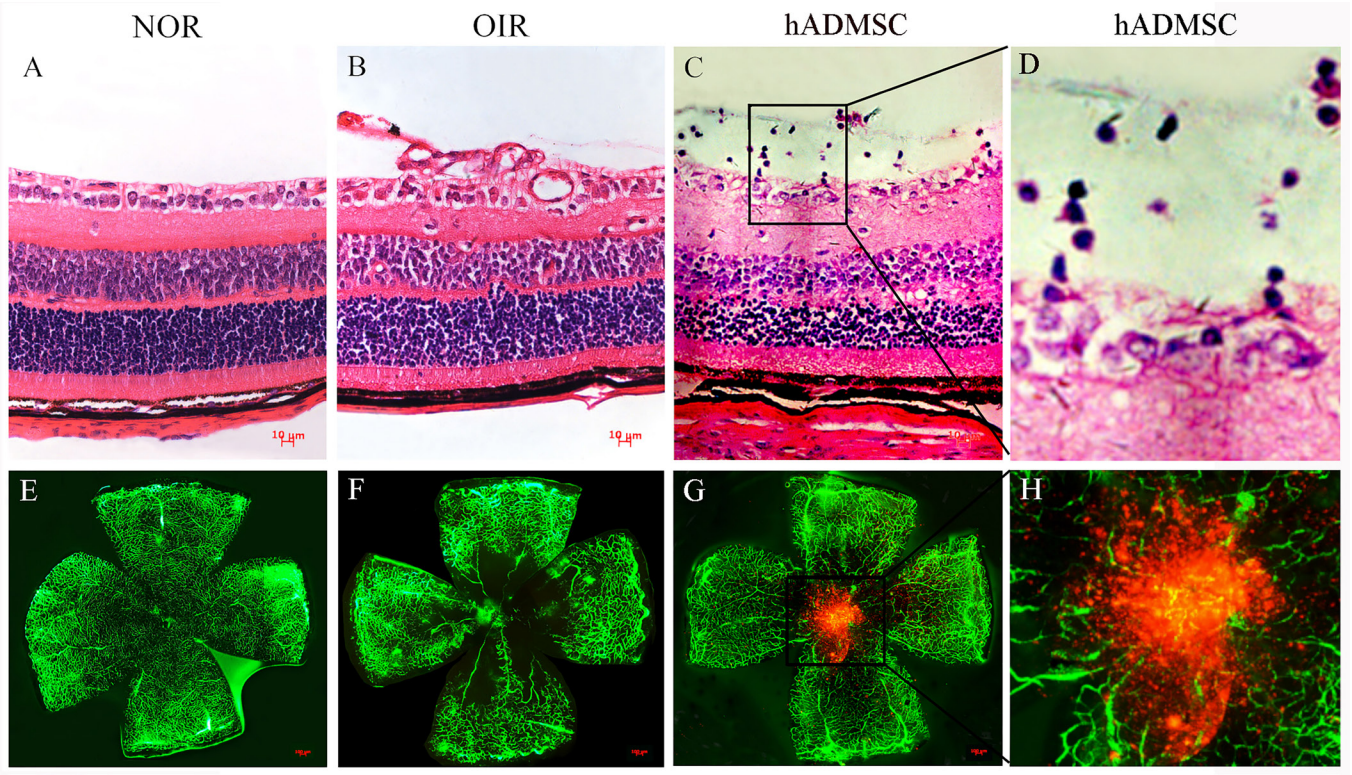

Figure 2. Intraocular tracking of hADSCs. **A**: The structure of each layer of the retina is clear, and there is no neovascularization breaking through the internal limiting membrane (ILM) in the P17 control group. **B**: Extensive neovascularization broke through the ILM in the P17 oxygen-induced retinopathy (OIR) group. **C**: No apparent neovascularization breaking through the ILM and the presence of injected cells above the ILM (not fused with the retina) are noted in the human adipose mesenchymal stem cells (hADSCs) injection group. **D**: Partial magnification of Figure 3C (30X). **E**: The retinal blood vessels are smooth and without neovascularization, and there is no perfusion area in the control group. **F**: There is extensive highly green fluorescent neovascularization in the periphery area and non-perfusion in the central area of the retina in the OIR group. **G**: Neovascularization and non-perfusion are significantly reduced in the hADSC injection group compared with that in the OIR group, and labeled hADSCs with red fluorescence are seen above the neovascularization and non-perfusion area. **H**: Partial magnification of Figure 3G (30X).

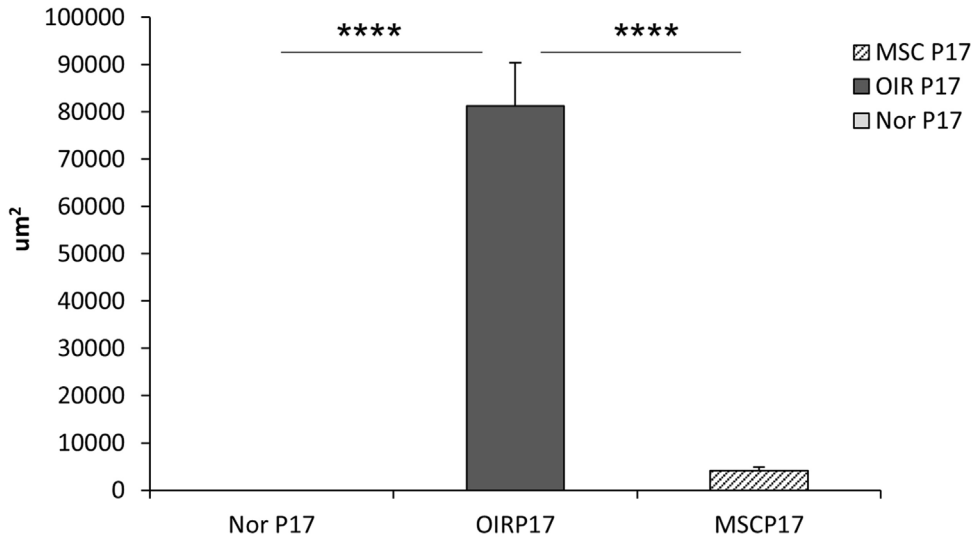

Figure 3. Quantification of retinal neovascularization. The neovascularization area of the oxygen-induced retinopathy (OIR) group is significantly increased compared to that of the normal group ($p<0.0001$), and the mesenchymal stem cell (MSC) group is significantly decreased compared to that of the OIR group ($p<0.0001$).

[32]. Studies have shown that the fate of intravitreally injected MSCs is integrated into the retina, or they remain in the vitreous cavity as cell clusters. A study showed no effects of intravitreally injected MSCs at 240 days after injection [33]. Metabolism after intraocular application requires further research. We used HE staining and electron microscopy to detect the proliferation, degeneration, and safety of ADMSCs in an intravitreal application. This study showed no noticeable retinal proliferation, deterioration, or apoptosis after ADMSC intravitreal application. There have been several reports related to the safety of MSC intraocular application. Leow et al. confirmed that intravitreally injected human Wharton's jelly-derived mesenchymal stem cells caused no side effects when applied in a retinal degeneration model [34]. However, in a clinical trial, a report showed three cases of vision loss after patients with AMD received bilateral intravitreal injections of autologous adipose tissue–derived stem cells [35]. This study did not find cell changes after the intravitreal application of hADSCs, which is Loew inconsistent with clinical reports. We speculate that different pathological environments and injection amounts of stem cells are the main reasons for the different proliferation outcomes.

A study showed that optic nerve cells degenerated under pathological conditions at 12 h [36]. In addition, Adi et al.

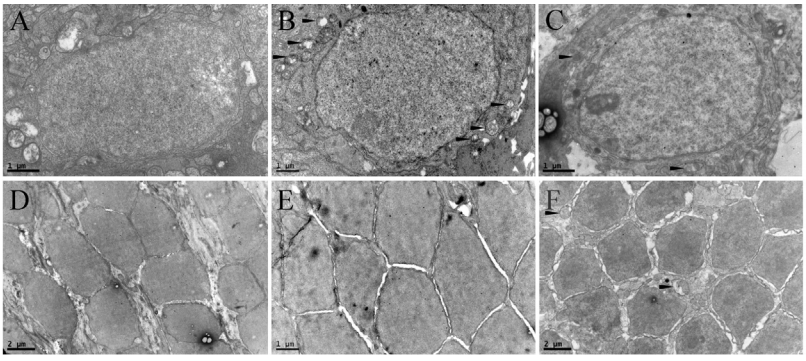

Figure 4. RGCs and cells of the outer nuclear layer are shown with electron microscopy. **A**, **D**: The retinal ganglion cell (RGCs) and the cells of the outer nuclear layer have a defined plasma membrane and uniformly distributed chromatin in the control group. **B**, **E**: There are many autophagosomes (black arrow) in the oxygen-induced retinopathy (OIR) group. **C**, **F**: There are fewer autophagosomes (black arrow) in the human adipose mesenchymal stem cell (hADSC) injection group than in the OIR and control groups. However, there is no noticeable difference in cells among the three groups.

reported that retinal proliferation was not observed in a rat model of retinal degeneration for up to 6 weeks [37]. One study showed, as studies have shown that less than 1% of ADMSCs express HLA-DR, which confirms that the antigenicity of human hADSCs is low [38], and that transplantation of allogeneic hADSCs does not cause an immune response. In addition, Sonia et al. showed no significant difference in eye, liver, spleen, or gonadal tissue between an MSC intravitreally injected group and a group without injection [39]. We still need to pay more attention to the risks in the application of MSCs and conclude the "safety" of other aspects, such as systemic side effects, daily behavior after transplantation, etc.

One of the limitations of this study is the short observation time for the safety of human adipose-derived stem cell application. The animal model is the main reason. The OIR model is a classic model that can simulate pathological retina neovascularization, but the model cycle time point is P12, and P17, 5 days. Although we used electron microscopy for safety, extended observation periods are needed in subsequent experiments. Another limitation is that we observed the cell expression characteristics only after induction in vitro. It will have more significance if we check the cell expression characteristics after intravitreal injection in vivo.

In conclusion, we provide preliminary theoretical support for the induction, distribution, fusion, proliferation, and safety of hADSCs in an intraocular application. However, there are still many challenges in the clinical intraocular application of MSCs. One challenge is the length and detail of the intraocular survival and function of MSCs. Furthermore, it is important to determine which MSCs are the best choice (MSCs, induced MSCs, or exosomes). Combining stem cells and extracellular vesicles and modifying host factors might be definitive therapy. More research is needed to clarify these questions.

APPENDIX 1. STR ANALYSIS.

To access the data, click or select the words "Appendix 1."

ACKNOWLEDGMENTS

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REFERENCES

1. Tang Y, Cheng Y, Wang S, Wang Y, Liu P, Wu H. Review: The Development of Risk Factors and Cytokines in Retinal Vein Occlusion. Front Med (Lausanne) 2022; 9:910600-[PMID: 35783660].
2. Zheng Y, He M, Congdon N. The worldwide epidemic of diabetic retinopathy. Indian J Ophthalmol 2012; 60:428-[PMID: 22944754].
3. Campochiaro PA. Retinal and Choroidal Vascular Diseases: Past, Present, and Future: The 2021 Proctor Lecture. Invest Ophthalmol Vis Sci 2021; 62:26-[PMID: 34817536].
4. Cheung N, Mitchell P, Wong T, Lancet Y. Diabetic retinopathy. Lancet 2010; 376:124-36. .
5. Jeffery RCH, Mukhtar SA, Lopez D, Preen DB, McAllister IL, Mackey DA, Morlet N, Morgan WH, Chen FK. Incidence of newly registered blindness from age-related macular degeneration in Australia over a 21-Year Period: 1996–2016. Asia-Pac J Ophthalmol 2021; 10:442-9. [PMID: 34534144].
6. Kodjikian L, Bellocq D, Mathis T. Pharmacological management of diabetic macular edema in real-life observational studies. BioMed Res Int 2018; 2018:8289253-[PMID: 30246026].
7. Nava MM, Raimondi MT, Pietrabissa R. Controlling self-renewal and differentiation of stem cells via mechanical cues. J Biomed Biotechnol 2012; 2012:797410-[PMID: 23091358].
8. Ding S, Schultz PG. A role for chemistry in stem cell biology. Nat Biotechnol 2004; 22:833-40. [PMID: 15229546].
9. Gaddam S, Periasamy R, Gangaraju R. Adult stem cell therapeutics in diabetic retinopathy. Int J Mol Sci 2019; 20:4876-[PMID: 31575089].
10. Park SS, Moisseiev E, Bauer G, Anderson JD, Grant MB, Zam A, Zawadzki RJ, Werner JS, Nolta JA. Advances in bone marrow stem cell therapy for retinal dysfunction. Prog Retin Eye Res 2017; 56:148-65. [PMID: 27784628].
11. Huang C, Luo W, Wang Q, Ye Y, Fan J, Lin L, Shi C, Wei W, Chen H, Wu Y, Tang Y. Human mesenchymal stem cells promote ischemic repairment and angiogenesis of diabetic foot through exosome miRNA-21–5p. Stem Cell Res (Amst) 2021; 52:102235-[PMID: 33601096].
12. Gong M, Yu B, Wang J, Wang Y, Liu M, Paul C, Millard RW, Xiao DS, Ashraf M, Xu M. Mesenchymal stem cells release exosomes that transfer miRNAs to endothelial cells and promote angiogenesis. Oncotarget 2017; 8:45200-12. [PMID: 28423355].
13. . Millán -Rivero JE. Nadal-Nicolás FM, García -Bernal D, Sobrado-Calvo P, Blanquer M, Moraleda JM, Vidal-Sanz M, Agudo-Barriuso M. Human Wharton's jelly mesenchymal stem cells protect axotomized rat retinal ganglion cells via secretion of anti-inflammatory and neurotrophic factors. Sci Rep 2018; 8:16299-[PMID: 30389962].
14. Satarian L, Nourinia R, Safi S, Kanavi MR, Jarughi N, Daftarian N, Arab L, Aghdami N, Ahmadieh H, Baharvand H. Intravitreal injection of bone marrow mesenchymal stem cells in patients with advanced retinitis pigmentosa; a safety study. J Ophthalmic Vis Res 2017; 12:58-[PMID: 28299008].
15. Qi Z, Zhang Y, Liu L, Guo X, Qin J, Cui G. Mesenchymal stem cells derived from different origins have unique sensitivities to different chemotherapeutic agents. Cell Biol Int 2012; 36:857-62. [PMID: 22694597].

16. Yu F, Ji S, Su L, Wan L, Zhang S, Dai C, Wang Y, Fu J, Zhang Q. Adipose-derived mesenchymal stem cells inhibit activation of hepatic stellate cells in vitro and ameliorate rat liver fibrosis in vivo. J Formos Med Assoc 2015; 114:130-8. [PMID: 25678175].

17. Pendleton C, Li Q, Chesler DA, Yuan K, Guerrero-Cazares H, Quinones-Hinojosa A. Mesenchymal stem cells derived from adipose tissue vs bone marrow: in vitro comparison of their tropism towards gliomas. PLoS One 2013; 8:e58198-[PMID: 23554877].

18. Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'amato R, Sullivan R, D'Amore PA. Oxygen-induced retinopathy in the mouse. Invest Ophthalmol Vis Sci 1994; 35:101-11. [PMID: 7507904].

19. Li S, Li T, Luo Y, Yu H, Sun Y, Zhou H, Liang X, Huang J, Tang S. Retro-orbital injection of FITC-dextran is an effective and economical method for observing mouse retinal vessels. Mol Vis 2011; 17:3566-[PMID: 22219652].

20. Schwartz SD, Regillo CD, Lam BL, Eliott D, Rosenfeld PJ, Gregori NZ, Hubschman J-P, Davis JL, Heilwell G, Spirn M. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. Lancet 2015; 385:509-16. [PMID: 25458728].

21. Nirwan RS, Albini TA, Sridhar J, Flynn HW Jr, Kuriyan AE. Assessing "cell therapy" clinics offering treatments of ocular conditions using direct-to-consumer marketing websites in the United States. Ophthalmology 2019; 126:1350-5. [PMID: 30904542].

22. Wang Y, Lv J, Huang C, Li X, Chen Y, Wu W, Wu R. Human Umbilical Cord-Mesenchymal Stem Cells Survive and Migrate within the Vitreous Cavity and Ameliorate Retinal Damage in a Novel Rat Model of Chronic Glaucoma. Stem Cells Int 2021; 2021:8852517-[PMID: 34733333].

23. Di G, Du X, Qi X, Zhao X, Duan H, Li S, Xie L, Zhou Q. Mesenchymal stem cells promote diabetic corneal epithelial wound healing through TSG-6–dependent stem cell activation and macrophage switch. Invest Ophthalmol Vis Sci 2017; 58:4344-54. [PMID: 28810264].

24. Ghazaryan E, Zhang Y, He Y, Liu X, Li Y, Xie J, Su G. Mesenchymal stem cells in corneal neovascularization: Comparison of different application routes. Mol Med Rep 2016; 14:3104-12. [PMID: 27514011].

25. Ezquer M, Urzua CA, Montecino S, Leal K, Conget P, Ezquer F. Intravitreal administration of multipotent mesenchymal stromal cells triggers a cytoprotective microenvironment in the retina of diabetic mice. Stem Cell Res Ther 2016; 7:1-17. [PMID: 26983784].

26. Castanheira P, Torquetti LT, Magalhãs DR, Nehemy MB, Goes AM. DAPI diffusion after intravitreal injection of mesenchymal stem cells in the injured retina of rats. Cell Transplant 2009; 18:423-31. [PMID: 19622229].

27. Johnson TV, Bull ND, Hunt DP, Marina N, Tomarev SI, Martin KR. Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. Invest Ophthalmol Vis Sci 2010; 51:2051-9. [PMID: 19933193].

28. Wen YT, Ho YC, Lee YC, Ding DC, Liu PK, Tsai RK. The Benefits and Hazards of Intravitreal Mesenchymal Stem Cell (MSC) Based-Therapies in the Experimental Ischemic Optic Neuropathy. Int J Mol Sci 2021; 22:2117-[PMID: 33672743].

29. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. Stem Cells Dev 2012; 21:2724-52. [PMID: 22468918].

30. Holan V, Hermankova B, Bohacova P, Kossl J, Chudickova M, Hajkova M, Krulova M, Zajicova A, Javorkova E. Distinct Immunoregulatory Mechanisms in Mesenchymal Stem Cells: Role of the Cytokine Environment. Stem Cell Rev Rep 2016; 12:654-63. [PMID: 27665290].

31. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci 2014; 15:4142-57. [PMID: 24608926].

32. Yin K, Wang S, Zhao RC. Exosomes from mesenchymal stem/stromal cells: a new therapeutic paradigm. Biomark Res 2019; 7:8-[PMID: 30992990].

33. Mesentier-Louro LA, Teixeira-Pinheiro LC, Gubert F, Vasques JF, Silva-Junior AJ, Chimeli-Ormonde L, Nascimento-Dos-Santos G, Mendez-Otero R, Santiago MF. Long-term neuronal survival, regeneration, and transient target reconnection after optic nerve crush and mesenchymal stem cell transplantation. Stem Cell Res Ther 2019; 10:121-[PMID: 30995945].

34. Leow S, Luu CD, Hairul Nizam M, Mok P, Ruhaslizan R, Wong H, Wan Abdul Halim WH, Ng M, Ruszymah B, Chowdhury S. Safety and efficacy of human Wharton's Jelly-derived mesenchymal stem cells therapy for retinal degeneration. PLoS One 2015; 10:e0128973-[PMID: 26107378].

35. Kuriyan AE, Albini TA, Townsend JH, Rodriguez M, Pandya HK, Leonard RE, Parrott MB, Rosenfeld PJ, Flynn HW Jr, Goldberg JL. Vision loss after intravitreal injection of autologous "stem cells" for AMD. N Engl J Med 2017; 376:1047-53. [PMID: 28296617].

36. Lampert PW, Vogel MH, Zimmerman LE. Pathology of the optic nerve in experimental acute glaucoma. Electron microscopic studies. Invest Ophthalmol 1968; 7:199-213. [PMID: 4966696].

37. Tzameret A, Sher I, Belkin M, Treves AJ, Meir A, Nagler A, Levkovitch-Verbin H, Rotenstreich Y, Solomon AS. Epiretinal transplantation of human bone marrow mesenchymal stem cells rescues retinal and vision function in a rat model of retinal degeneration. Stem Cell Res (Amst) 2015; 15:387-94. [PMID: 26322852].

38. Dam PTM, Hoang VT, Bui HTH, Hang LM, Hoang DM, Nguyen HP, Lien HT, Tran HTT, Nguyen XH, Nguyen Thanh L. Human Adipose-Derived Mesenchymal Stromal Cells Exhibit High HLA-DR Levels and Altered Cellular Characteristics under a Xeno-free and Serum-free Condition. Stem Cell Rev Rep 2021; 17:291-303. [PMID: 34510358].

39. Labrador Velandia S, Di Lauro S, Alonso-Alonso ML, Tabera Bartolomé S, Srivastava GK, Pastor JC, Fernandez-Bueno I. Biocompatibility of intravitreal injection of human mesenchymal stem cells in immunocompetent rabbits. Graefes Arch Clin Exp Ophthalmol 2018; 256:125-34. [PMID: 29168045].

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