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Functional Efficacy of Intravitreal Dental Pulp Stem Cells versus Human Umbilical Vein Endothelial Cell–Conditioned Media in Experimental Retinal Ischemia–Reperfusion Injury

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Objective: To evaluate the efficacy of intravitreal dental pulp stem cell–conditioned medium (DPSC-CM) versus human umbilical vein endothelial cell–CM (HUVEC-CM) in preserving retinal function after ischemia–reperfusion injury (IRI) in rabbits.

Design: Experimental animal study.

Subjects: Eighteen rabbits subjected to retinal IRI and randomized to receive intravitreal injections of DPSC-CM, HUVEC-CM, or balanced salt solution (BSS) (n = 6 per group).

Intervention: After induction of retinal IRI, rabbits received intravitreal injections of DPSC-CM, HUVEC-CM, or BSS. Electroretinography (ERG) was performed 7 days postinjection to assess retinal function, measuring both dark-adapted (DA) and light-adapted (LA) responses.

Main Outcome Measures: Preservation of a-wave and b-wave amplitudes on ERG as indicators of photoreceptor (PR) and bipolar cell function, respectively.

Results: Both DPSC-CM and HUVEC-CM significantly preserved b-wave amplitudes in DA ERG responses compared to BSS ($P < 0.05$). Dental pulp stem cell–conditioned medium demonstrated superior preservation of a-wave amplitudes, indicating enhanced PR function. Both treatments also maintained LA ERG responses. Transient mucopurulent discharge in 2 DPSC-CM–treated rabbits and a localized posterior subcapsular opacity in one BSS-treated rabbit were observed; all resolved without sequelae.

Conclusions: Intravitreal DPSC-CM and HUVEC-CM are effective in preserving retinal function after IRI, with DPSC-CM showing particular advantage in PR protection. These results support further investigation into DPSC-CM and HUVEC-CM as potential therapies for retinal ischemic conditions, such as retinal artery occlusion and anterior ischemic optic neuropathy.

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Ischemic injury of various organs in the body is a typically severe medical condition that often leads to significant debilitation or even death because of irreversible morphological and functional damage. Ischemia primarily affects mitochondrial membrane function. After reperfusion, the presence of oxygen and impaired mitochondrial function result in oxidative stress.[1] The accumulation of reactive oxygen species can cause damage to proteins and DNA in the cells, ultimately leading to apoptosis and programmed cell death.[2,3]

Tissues composed of terminally differentiated cells lack the ability to proliferate and replace damaged cells following ischemia–reperfusion injury (IRI). The retina, with its high oxygen demand and terminally differentiated neurons, is particularly vulnerable to ischemic damage. Recent evidence indicates that retinal IRI involves PANoptosis—a coordinated form of programmed cell death that integrates pyroptosis, apoptosis, and necroptosis—mediated in part by the activation of caspase-dependent pathways.[4]

Over the past decade, multiple cell therapy strategies have been explored for retinal and central nervous system ischemia. Mesenchymal stem cells (MSCs) derived from various sources such as bone marrow, adipose tissue, and umbilical cord have shown neuroprotective, anti-inflammatory, and angiogenic effects in preclinical IRI models. These benefits are largely mediated by paracrine signaling, with secretion of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor, and VEGF that promote neuronal survival, reduce apoptosis, and support vascular repair.[5,6] However, live cell

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therapies carry inherent risks, including immune rejection, uncontrolled differentiation, aberrant neovascularization, and tumorigenesis—concerns that are particularly relevant in ocular applications.[7] Furthermore, transplanted cells often demonstrate poor survival and limited integration into host tissue.[8] These limitations have driven increasing interest in cell-free approaches such as conditioned medium (CM), which can deliver the beneficial secretome of stem cells while avoiding the safety concerns associated with direct cell transplantation.[9]

In this study, we investigated the safety and functional efficacy (as revealed by electroretinography [ERG]) of conditioned media from 2 cell types with neurotrophic properties—dental pulp stem cells (DPSCs), which are multipotent stem cells, and human umbilical vein endothelial cells (HUVECs).

Our selection of HUVEC-CM and DPSC-CM was based on both mechanistic relevance and translational feasibility for retinal neuroprotection. Human umbilical vein endothelial cells secrete a neuroregenerative secretome enriched in VEGF, insulin-like growth factor 1, and BDNF, and their exosomes have been shown to attenuate neural apoptosis via microRNA-mediated pathways,[10] protect neural stem cells under ischemic conditions,[11] enhance Schwann cell–mediated nerve repair,[12] improve astrocyte function following ischemic stroke,[13] and promote neural stem cell expansion while preserving stemness.[14] Additional evidence supports their anti-inflammatory and anti-apoptotic properties[15] and neuroprotective effects in neonatal hypoxic–ischemia models.[16] In contrast, DPSCs, which are derived from the neural crest and exhibit natural affinity for neuronal and retinal lineages, produce CM rich in nerve growth factor, BDNF, and neurotrophin-3—factors critical for photoreceptor (PR) and retinal neuron survival.[17] Dental pulp stem cell-CM has outperformed bone marrow– and adipose-derived MSC-CM in neuroprotection and axonal regeneration,[18] demonstrated anti-inflammatory and neuroprotective effects in diabetic neuropathy models,[19] and preserved PR structure and function in retinal injury models.[17]

Although other MSC sources (eg, bone marrow) also possess neuroprotective potential,[20] DPSC-CM offers a particularly favorable neuroprotective profile for retinal lineages, whereas HUVEC-CM—especially after anti-VEGF modulation—provides neuroangiogenic benefits with readily scalable production. Together, these 2 CMs are expected to offer a rational and innovative approach to protecting retinal function after IRI.

Materials and Methods

Study Design

Eighteen male New Zealand White rabbits (average weight: 3000 g, age: 2 months) were housed in the Laboratory Animal Center, Shiraz University of Medical Sciences, Shiraz, Iran, in temperature-controlled rooms (20°C–22°C) under a 12-hour light/dark cycle. The rabbits were fed with a standard commercial chow diet ad libitum. Also, all efforts were made to minimize animal suffering during the experimental period. This study conforms to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocol was approved by the Ethical Committee at Shiraz University of Medical Sciences.

The animals were randomly divided into 3 groups. Group 1 (n = 6) received retinal IRI plus 0.1 mL of balanced salt solution (BSS), group 2 (n = 6) received IRI plus 0.1 mL of DPSC-CM, and group 3 received IRI plus 0.1 mL of HUVEC-CM. Only one eye (left eye) of each animal was included in the study for the induction of IRI. The right (untreated eye) was used as a negative control for ERG analysis.

Our rationale for selecting 6 animals per group was twofold. First, this sample size is routinely used in animal ophthalmology studies[21,22] and is considered sufficient for exploratory proof-of-concept experiments. Second, in line with the principles of the 3Rs (Replacement, Reduction, Refinement) and current ethical protocols, we were obliged to use the minimum number of animals necessary to address our study objectives while safeguarding animal welfare.

Surgical Procedure

At first, all rabbits were anesthetized with ketamine and xylazine (35 mg/kg and 5 mg/kg, respectively).[23] The left eyes were dilated with 0.5% tropicamide and 2.5% phenylephrine eye drops, followed by topical anesthesia with 0.5% tetracaine hydrochloride eye drops. Under aseptic conditions, and after the application of 5% povidone–iodine drops onto the ocular surface, a 23-gauge trocar was inserted 2.5 mm behind the limbus in the supratemporal quadrant.[24] Next, it was connected to a 500-mL BSS bottle. Intraocular pressure (IOP) was raised to 150 mmHg for 60 minutes (the ischemia period) by elevating the BSS bottle up to 205 cm over the rabbit's eye level (Fig 1A).[21] In each case, successful induction of retinal ischemia was confirmed by observing whitening of the retina with an indirect ophthalmoscope. At the end of the ischemia period, the bottle was lowered to eye level, and retinal reperfusion was observed. Next, the 23-gauge trocar was detached from the irrigating solution and 0.1 mL of BSS, DPSC-CM, or HUVEC (by randomization) was slowly injected through the trocar port into the intravitreal space of the eyes with a microsyringe fitted with a 27-gauge needle. Finally, the trocar was removed, the incision site was massaged with an applicator, and gentamicin ointment was applied at the conclusion of the surgery and q12 h thereafter for 4 days. During the entire surgery and for anesthesia recovery, the animals were kept on a heating pad. The right eyes were left untreated and served as negative controls.

Preparation of Stem Cell

Dental pulp stem cells were provided by the Stem Cell Technology Research Center at Shiraz University of Medical Sciences, Shiraz, Iran. These cells were cultured and characterized based on their transdifferentiation potential and the expression of MSC surface markers CD73 and CD105. Marker expression was evaluated using polymerase chain reaction (for primer sequences see Table 1), and the resulting products were assessed by gel electrophoresis to confirm DPSC identity. Under inverted microscope evaluation, the cells also exhibited the typical spindle-shaped morphology associated with DPSCs (Fig 2A).

Human umbilical vein endothelial cells (NCBI code: C554) were obtained from the Iranian National Cell Bank at the Pasteur Institute of Iran. The cells were cultured in Dulbecco's Modified Eagle Medium: F-12 (Shellmax) supplemented with 10% fetal

Table 1. Primer Sequences of DPSC Markers

Gene	Forward Primer	Reverse Primer	Amplicon Size
CD73	CAGCATTCCTGAAGATCCAAGC	TCCGTGTGTCTCAGGTTGTT	195
CD105	CCCATACCCAAAACCGGCAC	GACAGTCCTATGGACTTCCTGGTC	87

DPSC = dental pulp stem cell.

bovine serum (Gibco) and 1% penicillin-streptomycin (Shellmax). After initial seeding, the culture flasks were maintained in a CO_2 incubator with a humidified atmosphere at 37°C. Cells from passages 3 to 6 were used for the collection of CM (Fig 2B).

Because excessive VEGF in ophthalmic applications can promote inflammation, pathological neovascularization, and compromise the integrity of the inner blood–retinal barrier,[25] HUVECs—well recognized for their exceptionally high VEGF secretion [26,27]—were pretreated with 312.5-μg/mL bevacizumab (an anti-VEGF agent) for 24 hours. This concentration is equivalent to the intravitreal level achieved clinically when 1.25 mg of bevacizumab is injected into an average 4 mL human vitreous volume and has been shown to effectively neutralize VEGF while maintaining an acceptable safety profile. Our aim was to substantially reduce but not completely eliminate, free VEGF-A in the CM, thereby minimizing angiogenic risk while preserving potential VEGF-mediated neuroprotective effects. In contrast, although DPSC-CM also contains VEGF, its secretome exhibits a more balanced profile of angiogenic, anti-inflammatory, and neurotrophic factors and has been used safely in prior retinal studies without anti-VEGF modulation.[17,19]

After treatment, the cells were rinsed with phosphate-buffered saline and prepared for secretome collection.

Preparation of CM

To prepare DPSC-CM and HUVEC-CM, cells from passages 3 to 6 were seeded at a density of 8000 cells/cm^2. Upon reaching 80% confluence, the cells were washed 3 times with phosphate-buffered saline, and the medium was replaced with serum-free Dulbecco's Modified Eagle Medium (Gibco). After 72 hours, the medium was collected, centrifuged, and filtered through a 0.22-μm filter, then stored at −70°C until further use.

Outcome Measures

Primary Outcome Measure: Retinal Function. The acquisition of ERG in rabbits followed standard protocols discussed in previous reports.[5,23] In brief, after anesthesia and pupillary dilation (as previously described), trained operators, who were masked, performed standard scotopic (dark-adapted [DA]) and photopic (light-adapted [LA]) ERGs at 7 days postprocedure. The rabbits were DA for at least 1 hour and were anesthetized approximately 10 minutes prior to ERG recordings. Electroretinography waveforms were obtained using the RETIport system (Roland), in accordance with the International Society for Clinical Electrophysiology of Vision standards. We analyzed and recorded the following ERG parameters: DA 0.01 b-wave amplitude, DA 3.0 and 10.0 a- and b-wave amplitudes, LA 3.0 a- and b-wave amplitudes, LA 30-Hz flicker peak time, and oscillatory potential amplitude (Fig 1B, C).

Secondary Outcome Measure: Safety. All animals were also examined using slit lamp biomicroscopy and indirect ophthalmoscopy on days 1, 3, and 7 after injection to detect any complications such as uveitis, endophthalmitis, cataracts, etc.

Statistical Analysis

Data analysis was performed using SPSS, version 26, and MedCalc, version 20.008. Electroretinography parameters were compared among the 4 study groups: DPSC-CM (n = 6), HUVEC-CM (n = 6), BSS-treated positive controls (n = 6), and untreated fellow eyes without IRI induction (negative controls, n = 18). Group comparisons were conducted using the Kruskal–Wallis test, with a P value < 0.05 considered statistically significant. When overall significance was detected, post hoc pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni correction. Given 4 study groups, 6 pairwise comparisons were possible, and the Bonferroni adjustment set the corrected significance threshold at $\alpha = 0.05/6 \approx 0.0083$.

To balance the risks of type I and type II errors, we applied Bonferroni correction in pairwise testing to strictly control false-positives while also reporting nonsignificant P values <0.10 as trends when supported by consistent patterns across related ERG parameters.

Results

Retinal Function

Figure 3 depicts the results of ERG analysis for the 4 studied groups: DPSC-CM, HUVEC-CM, and BSS treatment of IRI (n = 6 for each treatment group) and control eyes (without IRI or treatment; n = 18). According to the Kruskall–Wallis test, the DA 0.01 b-wave amplitude, DA 3.0 a- and b-wave amplitudes, and DA 10.0 a-wave amplitude were significantly different between groups. The DA 10.0 b-wave analysis was also showed a nonsignificant trend ($P = 0.056$). Although the LA 3.0 a- and b-waves showed statistically significant differences, the LA 30-Hz flicker peak latency was not different between the studied groups. The same finding was observed for oscillatory potential amplitude.

Pairwise Comparisons. *With Negative Control Group.* The statistically significant differences between the 3 treatment groups of IRI-induced eyes and healthy control eyes (without IRI or treatment) are marked with an asterisk in Figure 3. The BSS-treated group had significantly lower a- and/or b-wave amplitudes compared to controls for DA 0.01, DA 3.0, DA 10.0, and LA 3.0 ERG responses. The DPSC-CM treatment group did not differ significantly from the control group in any of the evaluated responses, except for the LA 3.0 a-wave amplitude, which showed a trend toward a nonsignificant decrease ($P = 0.056$, Mann–Whitney U test). A weaker trend was also observed for DA 0.01 b-wave (Fig 3D; $P = 0.104$). For HUVEC-CM treatment eyes, the b-wave amplitudes of DA 3.0 and 10.0 responses were not different compared to the control group. However, the a-wave amplitudes of the

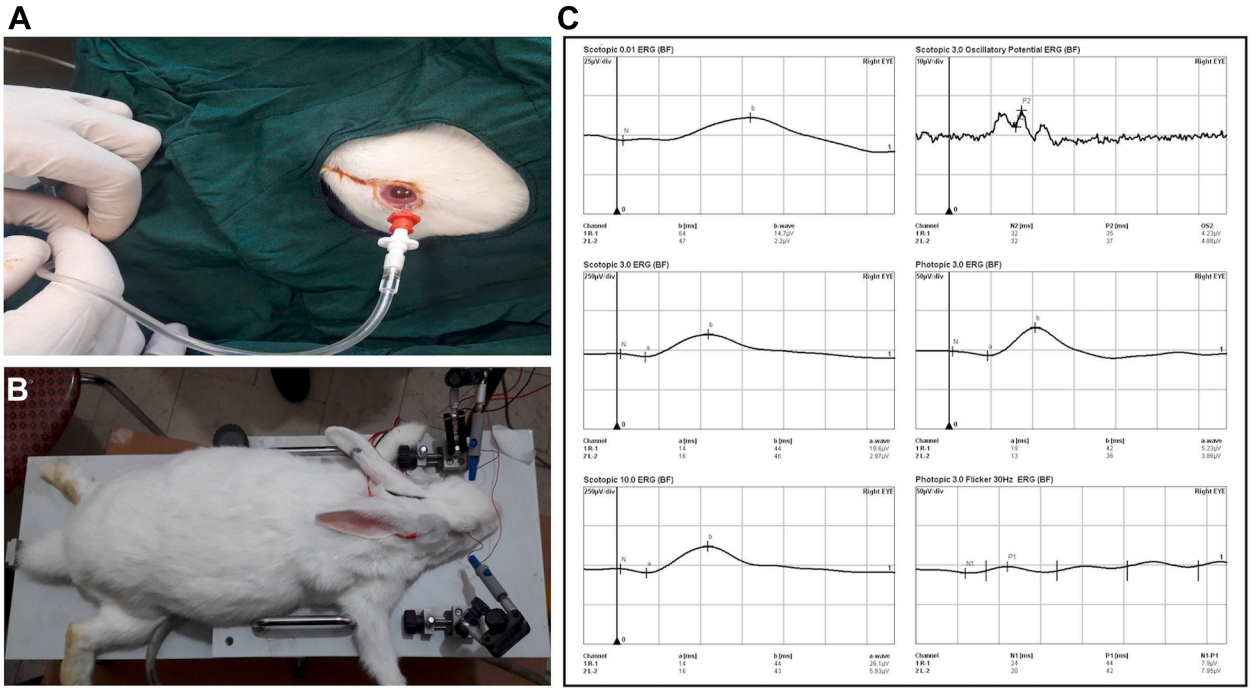

Figure 1. A, Illustration of the ischemia–reperfusion injury process induced by intraocular pressure elevation using a 23-gauge trocar attached to an irrigating balanced salt solution. **B,** Depiction of the ERG acquisition procedure from anesthetized albino rabbits. **C,** Representative ERG responses recorded from the right eye of a sample rabbit in our study. BF = bright flash; ERG = electroretinography.

mentioned responses were significantly lower with HUVEC-CM ($P = 0.003$ for both). The b-wave amplitude of DA 0.01 response was also lower than normal with a nonsignificant P value (0.056). In LA 3.0 response, the a-wave was not different from controls, but the b-wave was lower than normal ($P = 0.027$).

With Sham-Treatment (BSS) Group. In the DPSC-CM group, nonsignificant trends toward higher amplitudes compared with BSS-treated eyes were observed for the DA 3.0 a-wave and b-wave amplitudes ($P = 0.065$ for both) and the LA 3.0 b-wave amplitude ($P = 0.015$). No significant differences were detected in other ERG responses ($P \geq 0.180$). In the HUVEC-CM group, the DA 0.01 and DA 3.0 b-wave amplitudes both showed nonsignificant trends toward increase ($P = 0.026$ and $P = 0.093$, respectively). Other responses did not differ significantly from BSS-treated eyes ($P \geq 0.132$).

Between the 2 Treatment Groups (DPSC-CM vs. HUVEC-CM). Among all responses, the only difference between the 2 treatment groups was in the DA 3.0 a-wave amplitude (Fig 3A, left), with a nonsignificant P value of 0.093, where DPSC-CM showed better preservation than HUVEC-CM. All other responses showed no significant differences ($P \geq 0.132$).

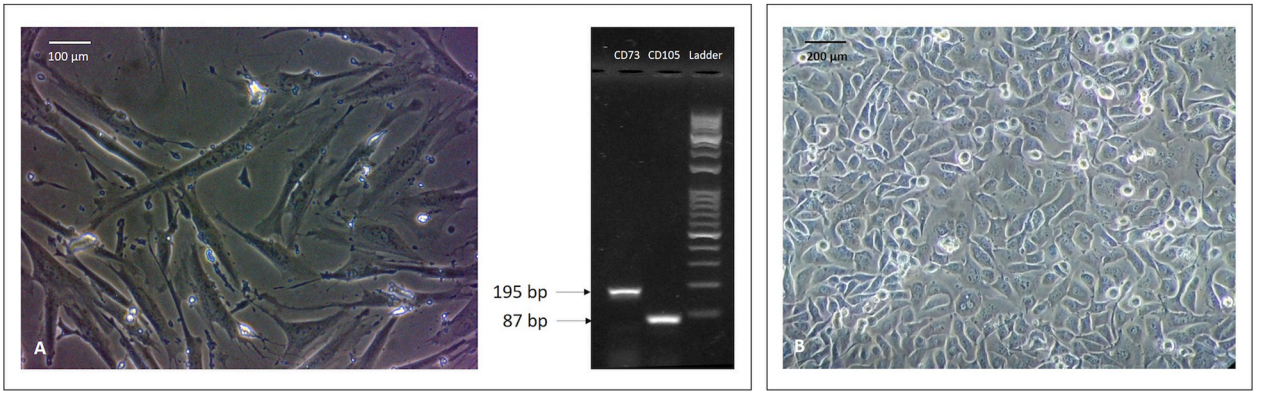

Figure 2. The typical morphology of **(A)** DPSCs observed under an inverted microscope at 400× magnification. The expression of CD73 and CD105 surface markers was confirmed by PCR and gel electrophoresis, as shown in the accompanying panel. **B,** Human umbilical vein endothelial cells observed under an inverted microscope at 200× magnification. DPSC = dental pulp stem cell; PCR = polymerase chain reaction.

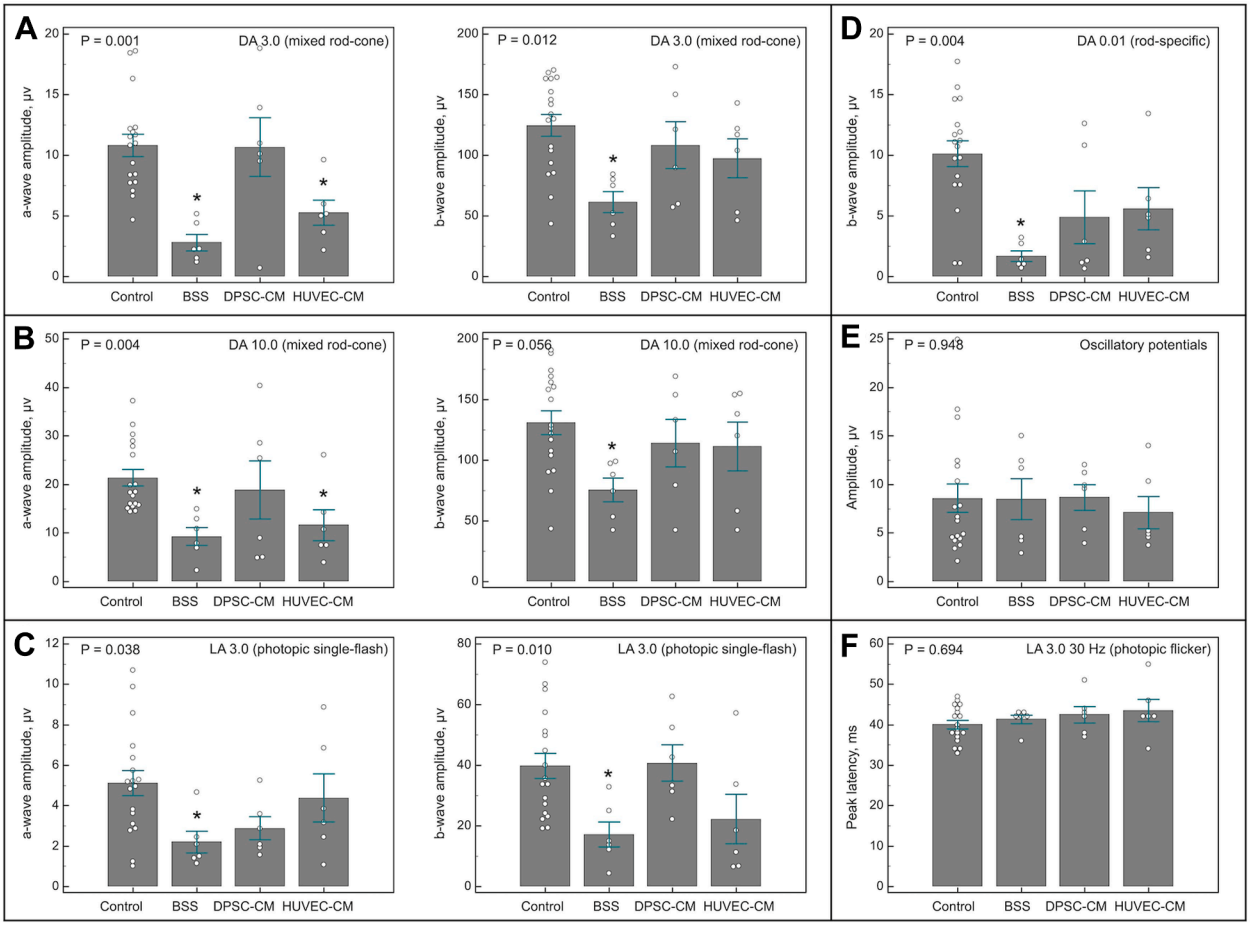

Figure 3. Comparison of DA and LA ERG responses among different treatment groups: DPSC-CM and HUVEC-CM (n = 6 each), the sham-treatment group (BSS; n = 6), and contralateral normal eyes without IRI or treatment (control; n = 18). Data are shown as mean values with error bars representing the 95% CI. *P* values were calculated using the Kruskal–Wallis test. An asterisk indicates a significant difference between the treatment groups and the normal untreated eyes (Control) based on pairwise comparison analysis using the Mann–Whitney *U* test with Bonferroni correction. **A,** DA 3.0 response; **B,** DA 10.0 response; **C,** LA 3.0 response; **D,** DA 0.01 response; **E,** oscillatory potentials; **F,** photopic flicker (30 Hz) response. BSS = balanced salt solution; CI = confidence interval; DA = dark-adapted; DPSC-CM = dental pulp stem cell–conditioned medium; ERG = electroretinography; HUVEC-CM = human umbilical vein endothelial cell-CM; IRI = ischemia–reperfusion injury; LA = light-adapted.

Safety

On the first day after the intravitreal injection, 2 rabbits in the DPSC-CM group exhibited mucopurulent discharge. Slit lamp examination revealed no anterior chamber reaction or media opacity indicative of endophthalmitis. In the BSS-treated group, one rabbit displayed a sectoral posterior subcapsular opacity at the site of trocar insertion. However, no anterior chamber reaction or vitritis was observed in this rabbit. Fundus examination yielded normal results for all 3 treatment groups.

On the third day after the injection, the mucopurulent discharge had resolved without any sequelae in the 2 aforementioned rabbits. Other examinations were unremarkable, and no signs of endophthalmitis, uveitis, or vitritis were detected in any group. On the seventh day of the study, both anterior and posterior segment examinations were completely normal in all groups.

Discussion

In this study, we found consistent results from different ERG responses supporting the protective effect of DPSC- and HUVEC-CMs in preserving retinal function after retinal IRI. Although both CMs were effective in maintaining the b-wave amplitude of DA responses, DPSC-CM outperformed HUVEC-CM in preserving DA a-wave amplitudes.

Dark-adapted a-waves and b-waves are primarily generated by PRs and bipolar cells, respectively.[28] Although retinal IRI mainly affects the inner retina, PRs are also compromised through retrograde mechanisms. Our findings suggest that both CMs were effective in preserving inner retinal function; however, DPSC-CM demonstrated an additional advantage in protecting PR function. These results align with previous studies that

provided both structural and functional evidence supporting the role of HUVEC and DPSC in maintaining neural cells, including those in the inner retina.[16,18,19,29–32] The protective effects of these CMs are largely attributed to their anti-inflammatory, neurotrophic, and proangiogenic growth factors.[19] Alsaeedi et al[17] demonstrated that DPSCs effectively protected PRs in a rat model of retinal degeneration, a finding corroborated by other studies.[22,33] This PR-specific preservation is believed to be mediated by growth factors such as nerve growth factor, BDNF, and neurotrophin-3, which are secreted in higher quantities in DPSC-CM.[17]

Our decision to use CM instead of stem cells was based on 3 key considerations. First, CM is a cell-free therapy, which minimizes the risk of inflammation and completely eliminates the potential for tumor formation—concerns commonly associated with cell-based therapies.[9,34] Second, CM can be conveniently administered in a reasonable volume (100 μL) via the intravitreal route, a standard procedure in retinal therapeutics. Lastly, studies using retinal ischemia models have shown that stem cells injected into the vitreous often remain unchanged, with their primary benefit attributed to the secretion of growth factors.[8,20] Therefore, the main objective of CM-based therapies is to protect neural cells during periods of stress, such as retinal IRI in our study. These interventions are most effective when administered at the earliest stages of the disease, as we have done.

We propose that the intravitreal injection of HUVEC-CM and, particularly, DPSC-CM holds promise as a therapeutic option for acute retinal ischemic conditions, such as branch and central retinal artery occlusions and anterior ischemic optic neuropathy. However, before advancing to clinical application, more extensive and long-term preclinical safety studies are required.

The method of IOP rise used in our study was in line with previous reports in terms of the level of IOP rise and duration of retinal IRI.[21] However, because the globe size in albino rabbits is close to that of humans, we used 23-gauge trocar insertion for both increasing IOP and delivering CM for the first time. In our experience, this was a simple and effective method and has the potential to become a standard method for retinal IRI induction in rabbits or other animals with human-sized globes.

Powers and Limitations

This study demonstrated the potential of stem cell–derived CM in mitigating ERG deficits after retinal IRI, a topic not previously reported. Several limitations should be acknowledged. The small sample size may have reduced statistical power, increasing the risk of a type II error (failing to detect a true effect); therefore, results with $P < 0.1$ were reported as nonsignificant trends when supported by consistent functional patterns across related ERG parameters. Also, to further compensate for this limitation, we pooled the untreated fellow eyes (n = 18) into a single negative control group, which increased the overall statistical power of comparisons. At the same time, the risk of type I error (false-positives) was minimized by applying Mann–Whitney U tests with Bonferroni correction for pairwise comparisons, ensuring strict statistical rigor despite limited group sizes. A methodological imbalance was introduced by pretreating HUVEC-CM with bevacizumab to neutralize its excessive VEGF output, whereas DPSC-CM was not modified; this approach was chosen to address a specific safety concern rather than to normalize angiogenic profiles of the 2 CMs. VEGF-A inhibition after bevacizumab pretreatment was not directly measured, which remains a methodological flaw. Another limitation is that the neurotrophic and anti-inflammatory components of the CMs were not directly quantified and our mechanistic interpretations were based on previously published reports.[10,11,18,19] Future studies should include quantitative and proteomic analyses to directly correlate CM composition with the observed neuroprotective effects. Finally, the short 7-day follow-up prevents assessment of long-term durability of the observed neuroprotective effects or delayed adverse events such as inflammation or cataract formation, highlighting the need for extended studies (eg, 4–8 weeks or longer) incorporating structural and biochemical analyses alongside functional outcomes.

Conclusions

The present proof-of-concept study showed that early intervention with stem cell–derived CM can preserve retinal function in retinal IRI injury. This finding is promising for the future development of treatment strategies for currently untreatable retinal conditions such as central and branch retinal artery occlusion and anterior ischemic optic neuropathy. According to our findings, although both DPSC- and HUVEC-CMs were effective in preserving DA b-wave amplitudes (a surrogate of bipolar cell functions), DPSC-CM performed better in preserving DA 3.0 and 10.0 a-wave amplitudes, suggesting a superior impact on PR preservation after retinal IRI. In this study, we did not encounter any clinical complications after CM injection up to 1 week after the procedure.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Footnotes and Disclosures

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All authors have completed and submitted the ICMJE disclosures form.

The authors have no proprietary or commercial interest in any materials discussed in this article.

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ANIMAL SUBJECTS: Animal subjects were used in this study. This study conforms to the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocol was approved by the Ethical Committee at Shiraz University of Medical Sciences.

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Data collection: Maalhagh, Sanie-Jahromi, Fazlinejad, Shahrivar, Farvardin

Analysis and interpretation: Nowroozzadeh

Obtained funding: N/A

Overall responsibility: Nowroozzadeh, Maalhagh, Sanie-Jahromi, Fazlinejad, Shahrivar, Farvardin

Abbreviations and Acronyms:
BDNF = brain-derived neurotrophic factor; **BSS** = Balanced Salt Solution; **CM** = conditioned medium; **DA** = dark-adapted; **DPSC** = dental pulp stem cell; **ERG** = electroretinography; **HUVEC** = human umbilical vein endothelial cell; **IOP** = intraocular pressure; **IRI** = ischemia−reperfusion injury; **LA** = light-adapted; **MSC** = mesenchymal stem cell; **PR** = photoreceptor.

Keywords:
Conditioned medium, Dental pulp stem cell, Electroretinography, Human umbilical cord endothelial cell, Ischemia−reperfusion injury.

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References

1. Fernandez DC, Bordone MP, Chianelli MS, et al. Retinal neuroprotection against ischemia-reperfusion damage induced by postconditioning. *Invest Ophthalmol Vis Sci*. 2009;50:3922−3930.
2. Oliva J. Therapeutic properties of mesenchymal stem cell on organ ischemia-reperfusion injury. *Int J Mol Sci*. 2019;20:5511.
3. Minhas G, Sharma J, Khan N. Cellular stress response and immune signaling in retinal ischemia-reperfusion injury. *Front Immunol*. 2016;7:444.
4. Yan WT, Zhao WJ, Hu XM, et al. PANoptosis-like cell death in ischemia/reperfusion injury of retinal neurons. *Neural Regen Res*. 2023;18:357−363.
5. Mathew B, Poston JN, Dreixler JC, et al. Bone-marrow mesenchymal stem-cell administration significantly improves outcome after retinal ischemia in rats. *Graefes Arch Clin Exp Ophthalmol*. 2017;255:1581−1592.
6. Ong JM, da Cruz L. A review and update on the current status of stem cell therapy and the retina. *Br Med Bull*. 2012;102:133−146.
7. Peng J, Zhang Z, Li M, et al. From adipose to action: reprogramming stem cells for functional neural progenitors for neural regenerative therapy. *Int J Mol Sci*. 2025;26:6599.
8. Guo Y, Saloupis P, Shaw SJ, et al. Engraftment of adult neural progenitor cells transplanted to rat retina injured by transient ischemia. *Invest Ophthalmol Vis Sci*. 2003;44:3194−3201.
9. Kichenbrand C, Velot E, Menu P, et al. Dental pulp stem cell-derived conditioned medium: an attractive alternative for regenerative therapy. *Tissue Eng B Rev*. 2019;25:78−88.
10. Jiang Y, Xie H, Tu W, et al. Exosomes secreted by HUVECs attenuate hypoxia/reoxygenation-induced apoptosis in neural cells by suppressing miR-21-3p. *Am J Transl Res*. 2018;10:3529−3541.
11. Zhou S, Gao B, Sun C, et al. Vascular endothelial cell-derived exosomes protect neural stem cells against ischemia/reperfusion Injury. *Neuroscience*. 2020;441:184−196.
12. Huang J, Zhang G, Li S, et al. Endothelial cell-derived exosomes boost and maintain repair-related phenotypes of schwann cells via miR199-5p to promote nerve regeneration. *J Nanobiotechnology*. 2023;21:10.
13. Gao X, Gao H, Yue K, et al. Observing extracellular vesicles originating from endothelial cells in vivo demonstrates improved astrocyte function following ischemic stroke via aggregation-induced emission luminogens. *ACS Nano*. 2023;17:16174−16191.
14. Zhang YZ, Liu F, Song CG, et al. Exosomes derived from human umbilical vein endothelial cells promote neural stem cell expansion while maintain their stemness in culture. *Biochem Biophys Res Commun*. 2018;495:892−898.
15. Larasati VA, Lembang GV, Tjahjono Y, et al. In vitro neuroprotective effect of the bovine umbilical vein endothelial cell conditioned medium mediated by down-regulation of IL-1β, Caspase-3, and Caspase-9 expression. *Vet Sci*. 2022;9:48.
16. Wu CC, Chen YC, Chang YC, et al. Human umbilical vein endothelial cells protect against hypoxic-ischemic damage in neonatal brain via stromal cell-derived factor 1/C-X-C chemokine receptor type 4. *Stroke*. 2013;44:1402−1409.
17. Alsaeedi HA, Koh AE, Lam C, et al. Dental pulp stem cells therapy overcome photoreceptor cell death and protects the retina in a rat model of sodium iodate-induced retinal degeneration. *J Photochem Photobiol B*. 2019;198:111561.
18. Mead B, Logan A, Berry M, et al. Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. *PLoS One*. 2014;9:e109305.
19. Makino E, Nakamura N, Miyabe M, et al. Conditioned media from dental pulp stem cells improved diabetic polyneuropathy through anti-inflammatory, neuroprotective and angiogenic actions: cell-free regenerative medicine for diabetic polyneuropathy. *J Diabetes Investig*. 2019;10:1199−1208.
20. Dreixler JC, Poston JN, Balyasnikova I, et al. Delayed administration of bone marrow mesenchymal stem cell conditioned medium significantly improves outcome after retinal ischemia in rats. *Invest Ophthalmol Vis Sci*. 2014;55:3785−3796.
21. Riazi-Esfahani M, Kiumehr S, Asadi-Amoli F, et al. Effects of intravitreal morphine administered at different time points after reperfusion in a rabbit model of ischemic retinopathy. *Retina*. 2009;29:262−268.
22. Lam C, Alsaeedi HA, Koh AE, et al. Human dental pulp stem cells (DPSCs) therapy in rescuing photoreceptors and establishing a sodium iodate-induced retinal degeneration rat model. *Tissue Eng Regen Med*. 2021;18:143−154.

23. Talebnejad MR, Sadeghi-Sarvestani A, Nowroozzadeh MH, et al. The effects of microwave radiation on rabbit's retina. *J Curr Ophthalmol*. 2018;30:74−79.
24. Afarid M, Lashkarizadeh H, Ashraf MJ, et al. The efficacy of intravitreal interferon alpha-2b for the treatment of experimental endotoxin-induced uveitis. *Indian J Ophthalmol*. 2016;64:376−381.
25. Ozaki H, Hayashi H, Vinores SA, et al. Intravitreal sustained release of VEGF causes retinal neovascularization in rabbits and breakdown of the blood-retinal barrier in rabbits and primates. *Exp Eye Res*. 1997;64:505−517.
26. Nigro E, Mallardo M, Polito R, et al. Adiponectin and leptin exert antagonizing effects on HUVEC tube formation and migration modulating the expression of CXCL1, VEGF, MMP-2 and MMP-9. *Int J Mol Sci*. 2021;22:7516.
27. Latifi-Navid H, Soheili ZS, Samiei S, et al. Network analysis and the impact of aflibercept on specific mediators of angiogenesis in HUVEC cells. *J Cell Mol Med*. 2021;25:8285−8299.
28. Weymouth AE, Vingrys AJ. Rodent electroretinography: methods for extraction and interpretation of rod and cone responses. *Prog Retin Eye Res*. 2008;27:1−44.
29. Hsu TW, Lu YJ, Lin YJ, et al. Transplantation of 3D MSC/HUVEC spheroids with neuroprotective and proangiogenic potentials ameliorates ischemic stroke brain injury. *Biomaterials*. 2021;272:120765.
30. Hu ZB, Chen HC, Wei B, et al. Platelet rich plasma enhanced neuro-regeneration of human dental pulp stem cells in vitro and in rat spinal cord. *Ann Transl Med*. 2022;10:584.
31. Gong P, Tian Q, He Y, et al. Dental pulp stem cell transplantation facilitates neuronal neuroprotection following cerebral ischemic stroke. *Biomed Pharmacother*. 2022;152:113234.
32. Mead B, Logan A, Berry M, et al. Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. *Invest Ophthalmol Vis Sci*. 2013;54:7544−7556.
33. Alsaeedi HA, Lam C, Koh AE-H, et al. Looking into dental pulp stem cells in the therapy of photoreceptors and retinal degenerative disorders. *J Photochem Photobiol B: Biol*. 2020;203:111727.
34. Amondarain M, Gallego I, Puras G, et al. The role of microfluidics and 3D-bioprinting in the future of exosome therapy. *Trends Biotechnol*. 2023;41:1343−1359.