Therapeutic potential of human umbilical cord–derived mesenchymal stem cells transplantation in rats with optic nerve injury

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Purpose: There are no effective treatments currently available for optic nerve transection injuries. Stem cell therapy represents a feasible future treatment option. This study investigated the therapeutic potential of human umbilical cord–derived mesenchymal stem cell (hUC-MSC) transplantation in rats with optic nerve injury. Methods: Sprague–Dawley (SD) rats were divided into three groups: a no-treatment control group (n = 6), balanced salt solution (BSS) treatment group (n = 6), and hUC-MSCs treatment group (n = 6). Visual functions were assessed by flash visual evoked potential (fVEP) at baseline, Week 3, and Week 6 after optic nerve crush injury. Right eyes were enucleated after 6 weeks for histology. Results: The fVEP showed shortened latency delay and increased amplitude in the hUC-MSCs treated group compared with control and BSS groups. Higher cellular density was detected in the hUC-MSC treated group compared with the BSS and control groups. Co-localized expression of STEM 121 and anti-S100B antibody was observed in areas of higher nuclear density, both in the central and peripheral regions. Conclusion: Peribulbar transplantation of hUC-MSCs demonstrated cellular integration that can potentially preserve the optic nerve function with a significant shorter latency delay in fVEP and higher nuclear density on histology, and immunohistochemical studies observed cell migration particularly to the peripheral regions of the optic nerve.

Key words: Flash visual evoked potential, mesenchymal cell, optic nerve injury, transplantation, umbilical cord

The human optic nerve is made up of the axons of 1.2 million retinal ganglion cells that mediate the electric signals from the retinal photoreceptors on receiving visual stimuli.[3] As with most central nervous system components, the optic nerve is susceptible to degeneration from genetic causes or damage from acquired causes due to the inexistence of spontaneous regeneration in neurons.[2]

Flash visual evoked potential (fVEP) is a noninvasive tool that measures the electrical signal conduction along the visual pathway.[3] The fVEP waveform represents several positive and negative deflections designed as P1, N1, P2, N2, and N3 peaks by Creel et al.[4] Any visual pathway abnormality will affect the appearance of the fVEP waveform.[3]

In rodent models of optic neuritis, latency delay of the N1, P1, and N2 peaks was reported to have a strong correlation with the reduction of myelination of the optic nerve. Similarly, the magnitude of P1 peak, either measured from N1 or N2 peak, was reported to have a strong correlation with the neuronal cell density of the optic nerve.[5]

Human umbilical cord–derived mesenchymal stem cells (hUC-MSCs) have been explored for potential cell-based therapies of various diseases such as ischemic stroke,[7] spinal cord injury,[8] Parkinson disease,[9] cardiovascular diseases,[10] myogenic disease,[11] and cornea-related diseases.[12]

This study aims to utilize an animal model of traumatic optic neuropathy by performing optic nerve crush injury in Sprague–Dawley (SD) rats to examine the therapeutic potential of treatment using hUC-MSC. The outcomes on optic nerve function are evaluated via fVEP and neuronal cell density following immunohistological analysis.

Methods

Animals

SD rats (n = 18; weighing between 300 and 350 g; age 10–14 weeks; Laboratory Animal Resource Unit, Kuala Lumpur, Malaysia) were used. All animals were maintained in an air-conditioned...
room with controlled temperature (21 ± 2 °C), fixed daily
12-hour light/12-hour dark cycles, and in individually ventilated
specific pathogen-free cages in an animal laboratory at the
Tissue Engineering Centre, Universiti Kebangsaan Malaysia
Medical Centre, Kuala Lumpur, Malaysia. The study was
approved by the institutional Animal Ethics Committee (AEC;
Approval number: FP/OFTAL/2012/MAE-LYNN/20-SEPT/462/
JAN2013-DEC2014). All procedures involving animals were
conducted in accordance with the guidelines drawn by the
institutional AEC and conformed to the ARVO (The Association
for Research in Vision and Ophthalmology) Statement for the
Use of Animals in Ophthalmic and Vision Research.

The animals were allocated to three groups (n = 6 per group).
Two control groups, namely, the negative control (Group A)
and the sham control (Group B) were employed with the
former receiving no treatment after the optic nerve injury
and the latter receiving an injection of Hank’s balanced saline
solution (HBSS) into the peribulbar space of the right eyes.
The treatment group (Group C) consisted of rats that received
treatment injections of a suspension of hUC‑MSCs cells into
the peribulbar space of the right eyes.

Each animal was anesthetized with 0.1 mL/kg bodyweight
of intramuscular injection of anesthesia regime containing
12.5 mg of tiletamine hydrochloride (Zoletil-50, Virbac Lab,
France), 12.5 mL of xylazine hydrochloride (Xylazil-20,
Tryo Lab, Australia), and 2.5 mL of ketamine hydrochloride
(Bioketan, Vetoquinol Biowet, Poland). The fVEP was measured
before optic nerve crush, 3 weeks after treatment, and 6 weeks
after treatment. After 6 weeks, the rats were sacrificed for
histological analysis [Fig. 1].

The rats were excluded when any complications that could
interfere with vision were present. The complications included
hemorrhage, endophthalmitis, or media opacities such as
cataract. Rats unfit to undergo anesthesia, those with a gross
physical abnormality, those with only one functional eye, those
having flat or unrecordable optic nerve function on fVEP, or
those that demonstrated histological evidence of postmortem
degradation were also excluded.

Stem cell preparation

The hUC‑MSC used were obtained from a local stem cell
bank (Cryocord™, Malaysia), which prepares the stem cells
in a good manufacturing practice–accredited laboratory. The
cells were subjected to evaluation according to the International
Society for Cellular Therapy criteria for mesenchymal stem
cell. Additionally, they were also tested for differentiation
capacity into neurons. Isolation of the mesenchymal stem cells
has been described previously.13 The method of obtaining and
preparing the stem cells has also been described in detail by
Leow et al.14 in their 2015 article.

The cells were cultured in a medium (low glucose Dulbecco’s modified Eagle’s medium; 10% human serum; 100
U/mL penicillin; 100 µg/mL streptomycin; 0.25 µg/mL
amphotericin; Gibco, USA). The cells were expanded until
reaching an appropriate density at passages 4 to 5, and used
throughout the experiment.

fVEP

Baseline visual function was evaluated by fVEP before crushing
the optic nerve. Following the optic nerve crushing, fVEP
was measured again at Week 3 and Week 6 after hUC‑MSCs
transplantation. The fVEP recording was done under full
anesthesia.

ROLAND RETI-port (Roland Consult, Brandenburg,
Germany) visual electrophysiology system with platinum
needle electrodes was used. The recording needle electrode
was placed at the rat’s occipital tuberosity, and the reference
needle electrode was inserted at the central frontal region, as
shown in Fig. 2a. The ground needle electrode was placed at
the animal’s ear.

Full visual field white flash stimulation was applied, with
a flash intensity of 3.93 cd/m² without background light and
with a stimulation frequency of 2 Hz, a band pass width of
1 to 100 Hz, and at 20,000 × magnification. The time for each
sampling was 25 ms, and the waveform was superimposed
50 times. The VEP for every rat was recorded three times at an
interval of 10 minutes. Data were collected for latency at N1, P1,
N2, and amplitude of N1‑P1 and P1‑N2 as depicted in Fig. 2b.

Optic nerve crush

Following measurement of baseline fVEP, the right eye optic
nerve was crushed while the contralateral eye acted as a
control. All operations were performed under full anesthesia.
Prior to the procedure, 0.5% proparacaine hydrochloride
(Alcon Laboratories, Fort Worth, USA) was applied to the eyes
for topical anesthesia, and 5% povidone (Alcon Laboratories,
Inc.) was used as an antiseptic to prevent infection.

The rats were placed in the lateral decubitus position during
the procedure. A small incision was made under a binocular
microscope with spring scissors over the temporal conjunctiva.
Care was taken on the depth of incision to avoid cutting into
the underlying musculature (lateral rectus, inferior rectus,
and inferior oblique muscles). With microforcesps, the edge of
the conjunctiva next to the globe was grasped and retracted,
rotating the globe nasally.

The exposed posterior aspect of the globe allows optic
nerve visualization. Retrobulbar tissues were further retracted
to expose the optic nerve. The optic nerve was clamped for
7 seconds using anatraumatic vascular clip (60 g microvascular
clip, World Precision Instruments, FL, USA) 2.0 mm behind the
eyeball to cause optic nerve injury. The vascular clip applied
a constant and consistent force on the optic nerve. The clip was
then released and removed after 7 seconds, thus allowing the
eye to rotate back into place.

Maxitrol™ (neomycin and polymyxin B sulfates and
dexamethasone, Novartis, Switzerland) eye ointment was used
after the operation to avoid infection. The rats were then placed
on a warm pad and monitored until they had fully recovered
from anesthesia. Subsequently, rats were monitored and given
Maxitrol™ eye drops every 6 hours, Maxitrol™ ointment at night,
and analgesics (oral ibuprofen continuously in water, 15 mg/kg/
day) for 1 week. The rats were monitored after the procedures
for possible complications, including infection and bleeding.

Stem cell injection

After the right eye optic nerve crush was performed, treatment
injections of hUC‑MSC cells suspension into the peribulbar
space of the right eyes were administered (1 × 10⁷ cells,
100 µL per eye). The injection was performed using an insulin
syringe (30G) through the inferotemporal quadrant, passing
through the conjunctiva, between the lateral third and the medial two thirds of the inferior orbital edge. It was positioned almost parallel to the orbital floor and advanced to 8 to 10 mm in depth. Cyclosporine-A (Bioshop, Canada) was administered through drinking water (210 mg/L) resulting in a blood concentration of 250-300 µg/L, which was given 2 days before cell injection. This immunosuppressive agent that reduces rejection of the transplanted cells has to be given ahead of the transplant, because it does not work immediately and takes some time to work.

Animals with complications from the peribulbar injection such as hemorrhage, endophthalmitis, and cataract were excluded from the study. The hUC-MSCs were obtained from the same supplier in the same batch, and all peribulbar injections were carried out by the same researcher to minimize variability.

**Histological analysis of the optic nerve**

The rats were sacrificed after fVEP recording under anesthesia at Week 6 using a lethal dose of sodium pentobarbital (100 mg/kg bodyweight) given intraperitoneally. The eyes were enucleated with complete dissection of the optic nerve. The eyes were then immersed in 2% paraformaldehyde for 1 hour, infiltrated with sucrose. The eyeballs with about 5 to 7 mm of the optic nerve were harvested and embedded in a paraffin
block for histology studies. Coronal sections were done with a microtome machine. The optic nerve was cut 2 mm away from the globe for a length of 1 mm. This portion of the optic nerve was then cut into multiple cross-sectional slices with a thickness of 3.0 µm before tissues were stained with hematoxylin and eosin (HE) for histological analysis.

Digital images were obtained at 10 × magnification using a light microscope (Olympus BX40; Olympus Optical Co. Ltd., Tokyo, Japan), and the histological examination of axonal density was done using software Q Capture Pro Version 5.1. Axonal density was calculated from the central region and the peripheral region of the optic nerve as shown in Fig. 2c.

Axons reviewed as nonviable had visibly long swollen axons, shrunken axons or axons with layer splitting of myelin sheaths, and fibrotic axons. Two different examiners counted the number of viable axons and compared it between the groups.

**Immunohistological staining**

A 4 µm thick tissue was fixed in 4% paraformaldehyde before being immersed in a preheated target retrieval solution and blocked with 10% goat serum to prevent nonspecific binding sites. Then the tissues were stained with primary mouse monoclonal antibody STEM121 (U.S. Stem Cell Inc., Florida, USA) specific for a human cytoplasmic marker to highlight the hUC-MSCs, and anti-S100 beta antibody (BD Biosciences, NJ, USA), which was a glial tissue-specific protein only expressed by a subtype of mature astrocytes. STEM121 specifically stains human protein, whereas anti-S100B antibody stains both human and rat proteins.

Primary antibody was detected by Goat anti-Mouse IgG Secondary Antibody, Alexa Fluor 488 (Life Technologies, USA) for anti-S100B antibody and Goat anti-Mouse IgG Secondary Antibody, Alexa Fluor 594 (Life Technologies) for STEM121. 4,6-Diamidino-2-phenylindole was used for nuclear staining. The sections were examined using fluorescence microscopy (Nikon, Japan).

**Statistical analysis**

Statistical analysis was performed using SPSS software (Version 19.0; IBM, Chicago, IL, USA) presented as the median ± interquartile range (IQR). Kruskal–Wallis and
Figure 3: Example of VEPs representative in Group B and Group C rats

Mann–Whitney U test were used to compare the outcome between independent groups. A P value <0.05 indicated a statistically significant difference.

Results

Following optic nerve crush, all the rats from all groups survived without any presentation of infection or tumor growth. However, one rat from the sham control group and four rats from the stem cell treatment group were excluded from the analysis following the exclusion criteria outlined. Two rats were excluded from the stem cell treatment group due to the presentation of hemorrhage during the optic nerve crush procedure. One rat from the sham control group and two rats from the stem cell treatment group were excluded from the analysis for having histological evidence indicative of postmortem degradation. This includes extensive tissue swelling, fibrotic axons, and shrunken axons with layer splitting of myelin sheaths, which made them unsuitable for data analysis.

Fig. 3 shows a representative fVEP trace obtained from rats in the sham control group and the stem cell treatment group before the optic nerve crush, as well as 3 and 6 weeks after treatment. The fVEP traces from the negative control group were excluded from the analysis following a noticeable difference between its baseline fVEP parameter values compared with

Figure 4: Comparison of baseline parameters of (a) latency of N1, P1, and N2; (b) amplitude N1-P1 and N2-P1 between no treatment control, sham treatment control, and stem cell groups. Kruskal–Wallis test revealed a significantly higher N1-P1 amplitude in no treatment group compared with the other two groups (*P < 0.05)
the rest of the experimental groups [Fig. 4]. In particular, the negative control group showed significantly higher N1-P1 amplitude compared with the other two groups ($P = 0.039$).

When latency values were plotted, statistically significant differences were noted in the latency parameters of N1 ($P = 0.015$) and P1 ($P = 0.041$) when comparing between the two groups at 6 weeks after treatment [Fig. 5a-c]. However, none of the differences in the latency values for N2 latency at 3 weeks after treatment or for all latency values at 6 weeks after treatment were statistically significant between the two groups.

In terms of amplitude, there was a significant deterioration of N1-P1 values in the stem cell treatment group ($P = 0.0036$) from baseline compared with after 3 weeks of treatment [Fig. 5d]. Alternatively, significant deterioration of N2-P1 amplitude values was observed in the sham control group ($P = 0.0036$) from baseline to 3 weeks after treatment [Fig. 5e].

**Histological outcomes**

Histopathology of optic nerve crush areas was observed with H and E. The specimens were found to be highly cellular on H and E staining. In the BSS treated group, as shown in Fig. 6a and b, there were more circumscribed areas of fibrotic axons and shrunken axons with splitting in the layers of myelin sheaths. These were areas of vacuolation and optic nerve edema with swollen axons. However, in the group treated with stem cells as shown in Fig. 6c and d, there was an increase in the cellular density within the injured axonal tissue, and at areas surrounding the injured optic nerve.
Cellular density counts were lower in the BSS treatment Group B compared with the stem cell treatment Group C, with the representative images of each group with center and peripheral counts shown in Fig. 6e. The mean cellular density counts were higher at 418.6 cells at the center area and 440.6 cells at the peripheral area following optic nerve injury treatment in the stem cell treatment group. In comparison, the BSS treatment Group B had cellular density counts of 209.3 cells at the center area and 295.2 cells at the peripheral area. There is a statistically significant difference between BSS treatment group and stem cell treatment group in terms of center and peripheral mean cellular density counts ($P = 0.001$ and $P = 0.038$, respectively).

The fluorescence microscopy images shown in Fig. 7a depict the localization of hUC-MSCs at Week 6 within the injured optic nerve. The hUC-MSCs appear to have survived and migrated to the peripheral and central regions of the injured optic nerve. Higher hUC-MSC density was seen at the peripheral regions of optic nerve injury compared with the central region.

In terms of neural differentiated cells, anti-S100B stained cells were found to prominently reside in the peripheral regions of the optic nerve injury [Fig. 7b]. This suggested there had been a differentiation of hUC-MSCs into glial tissues and mature astrocytes, as indicated by the peripheral region having a higher density of hUC-MSCs compared with the central region.

**Discussion**

From this study, it was observed that the hUC-MSCs showed potential as a therapy for optic nerve injury in a rat model. This study has demonstrated significant functional improvement following hUC-MSC transplant on fVEP, in some parameters in latency, although the same was not observed in the amplitude of the response. Furthermore, hUC-MSCs showed better histological evidence of cell recovery than saline.

The effects observed could be attributed to the potential anti-inflammatory effects of hUC-MSC transplantation, whereby inflammatory responses that occur when there is an injury are being controlled. Hence, the reduction in inflammation that results aids in the repair process and further triggers cell repair and optic nerve regeneration. Furthermore, the absence of significant reduction of fVEP parameters suggests that there is potential maintenance of visual function with stem cell treatment. However, further studies are warranted as longer follow-up, increased frequency of stem cell transplantation, and increased quantity of stem cells may optimize stem cell function in optic nerve regeneration.

Although the peribulbar route of administration chosen in this study has the advantage of direct implantation to the site of injury where the injured neurons are targeted and systemic spread was minimized, the dosage for injection of hUC-MSCs could still be inadequate. A higher dose could be needed as peribulbar transplantation of stem cells may have caused a distribution of the cells throughout the peribulbar space leading to a lower concentration in the subretinal space. Hence, repeated injections with transplantation of stem cells with longer follow-up duration could be done in the future studies to look for the optimal dosage needed for functional efficacy. Alternatively, future studies may consider a different site for stem cell delivery such as injection of stem cells directly into the optic nerve.

Second, the minimal changes in visual function observed could be due to fVEP in the study being not sensitive enough to detect the changes, and hence revealing inconsistent results. An
Figure 7: Fluorescence microscopy of (a) STEM121 at Week 6 in Group B and C. Scale bar represents 100 µm. STEM121 antibody marker (red) for mesenchymal stem cells and counterstained with DAPI (blue) to label the nucleus. Merged immunofluorescence image revealed co-localization of nuclei-positive cells, DAPI with STEM121. Arrows (peripheral) and arrowheads (center) indicate double-labeled cell areas. There was absence of STEM121 antibody expression in Group B. Labeled nuclei were seen to be more centrally located with some not expressing STEM121. (b) S100B at Week 6 in Groups B and C. Scale bar represents 100 µm. S100B antibody marker (green), which was glial specific, was used to stain mature astrocytes and counterstained with DAPI (blue) to label the nucleus. Merged immunofluorescence image revealed co-localization of nuclei-positive cells, DAPI with S100B antibody. Arrows (peripheral) and arrowheads (center) indicate double-labeled cell areas. Anti-S100B antibody expressed at the peripheral region. Labeled nuclei were seen to be more centrally located with some not expressing S100B.
fVEP performed through mini-Ganzfeld and screw electrodes could give better and more persistent results as shown by You et al.\textsuperscript{10} Besides, other electrophysiological tests associated with macula function such as pattern electroretinogram or multifocal electroretinogram that allow improved interpretation of an abnormal VEP could be done in the future studies. This would have allowed us to evaluate all the levels of electrophysiological response to determine the point at which pathology interfered with the signal. Unfortunately, due to cost and equipment issues, they could not be performed in this study.

Despite these limitations, histological analysis was able to demonstrate signs of early recovery. Still, the data were too small for statistical calculation. Stem cell treatment showed higher cellular density in the surrounding the optic nerve and intra-axonal regions, and improved optic nerve fibrosis, edema with vacuolation, and shrunken axons with layer splitting of the myelin sheath that were observed with HBSS treatment.

Detection of higher cellular density human STEM121 at the peripheral as well as central areas of optic nerve suggests that hUC-MSCs have the potential to differentiate into axon-like cells \textit{in vivo}. Similarly, anti-S100B stained glial tissues and mature astrocytes were also found at higher cellular density in the peripheral and central regions. These findings further suggest that the hUC-MSCs have the potential to differentiate into axonal-like cells \textit{in vivo}. Other studies have reported successful \textit{in vitro} induction of hUC-MSCs into neurons and glia,\textsuperscript{[15]} and retinal progenitor cells.\textsuperscript{[16]} This showed the potential of hUC-MSCs to differentiate into different cell lineages including cells that constitute optic nerve.

Although this pilot study has many limitations, the potential of hUC-MSC as a cell therapy for optic nerve injury warrants further studies with a longer duration of follow-up that can demonstrate neuronal synapses with an improvement of visual function.

### Conclusion

In conclusion, peribulbar transplantation of hUC-MSCs demonstrated cellular integration that can potentially preserve the optic nerve function as shown by the significantly shorter latency delay in fVEP, histological changes of higher nuclear density, and immunohistochemistry with STEM121 and anti-S100B antibody studies observed in areas of higher nuclear density, both in the central and peripheral regions of the optic nerve.

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### Conflicts of interest

There are no conflicts of interest.

### References

1. Harman A, Abrahams B, Moore S, Hoskins R. Neuronal density in the human retinal ganglion cell layer from 16-77 years. Anat Rec 2000;260:124–31.
2. Benowitz LI, Yin Y. Optic nerve regeneration. Arch Ophthalmol 2010;128:1059–64.
3. Paja-Wilczek D, Maruszczyzk W, Sirek S. Flash visual evoked potentials (fVEP) in various stimulation conditions. Doc Ophthalmol 2019;138:35–42.
4. Creel D, Dustman RE, Beck EC. Intensity of flash illumination and the visually evoked potential of rats, guinea pigs and cats. Vision Res 1974;14:725–9.
5. Van Der Walt A, Kolbe S, Mitchell P, Wang Y, Butzkueven H, Egan G, et al. Parallel changes in structural and functional measures of optic nerve myelination after optic neuritis. PLoS One 2015;10:e0121084. doi: 10.1371/journal.pone.0121084.
6. You Y, Klistorner A, Thie J, Graham SL. Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. Invest Ophthalmol Vis Sci 2011;52:6911–8.
7. Hess DC, Borlongan CV. Cell-based therapy in ischemic stroke. Expert Rev Neurother 2008;8:1193–201.
8. Yang CC, Shih YH, Ko MH, Hsu SY, Cheng H, Fu YS. Transplantation of human umbilical mesenchymal stem cells from Wharton’s jelly after complete transection of the rat spinal cord. PLoS One 2008;3:e3336. doi: 10.1371/journal.pone.003336.
9. Weiss ML, Medickey S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, et al. Human umbilical cord matrix stem cells: Preliminary characterization and effect of transplantation in a rodent model of parkinson’s disease. Stem Cells 2006;24:781–92.
10. Breymann C, Schmidt D, Hoerstrup SP. Umbilical cord cells as a source of cardiovascular tissue engineering. Stem Cell Rev 2006;2:87–92.
11. Conconi M, Burra P, Di Liddo R, Calore C, Turetta M, Bellini S, et al. CD105(+) cells from Wharton’s jelly show \textit{in vitro} and \textit{in vivo} myogenic differentiative potential. Int J Mol Med 2007;18:1089–96.
12. Ye J, Lee SY, KooK KH, Yao K. Bone marrow-derived progenitor cells promote corneal wound healing following alkali injury. Graefes Arch Clin Exp Ophthalmol 2008;246:217–22.
13. In ‘t Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 2004;22:1338–45.
14. Leow SN, Luu CD, Hairul Nizam MH, Mok PL, Ruhaslizan R, Wong HS, et al. Safety and efficacy of human wharton’s jelly-derived mesenchymal stem cells therapy for retinal regeneration. PLoS One 2015;10:e0128973. doi: 10.1371/journal.pone.0128973.
15. Bilic G, Zeisberger SM, Mallik AS, Zimmermann R, Zisch AH. Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. Cell Transplant 2008;17:955–68.
16. Koike-Kiriyama N, Adachi Y, Minamino K, Iwasaki M, Nakano K, Koike Y, et al. Human cord blood cells can differentiate into retinal nerve cells. Acta Neurobiol Exp (Wars) 2007;67:359–65.