Perspective

Exploring the potential application of dental pulp stem cells in neuroregenerative medicine

Nessa Sultan*, Ben A. Scheven

The trigeminal nerve and its peripheral branches are susceptible to injury in the dental practice due to surgical removal of impacted third molars and placement of dental implants. Although peripheral trigeminal nerve injuries can undergo spontaneous regeneration, some injuries may be permanent with varying degrees of continued sensory impairment and neuropathic pain ranging from mild numbness to complete anesthesia (Tay and Zuniga, 2007). Adult neurons require continued neurotrophic support from surrounding cells to sustain neural viability, inhibit death-inducing pathways activating a variety of cell survival pathways (Zheng and Quirion, 2004).

Stem cell therapies for neural repair and regeneration are rapidly advancing as they can function as an important source for trophic factors. Dental pulp stem cells (DPSC), which are isolated from the central pulpal core of teeth, have shown promise for neuroregenerative properties in various in vitro and in vivo experimental studies (Mead et al., 2017; Mohan and Ramalingam, 2020). DPSC can relatively easily be harvested from extracted teeth having the distinction, that they are derived from neural crest cells and are considered closer to neuronal lineages compared to other sources of mesenchymal stem/stromal cells (MSC) such as bone marrow-derived MSC (Sakai et al., 2012). DPSC have been reported to differentiate along neural lineages when treated with neurotrophic growth factors such as nerve growth factor (NGF), epidermal growth factors, retinoic acid, fibroblastic growth factor (Ovindasamy et al., 2010). With this inherent capacity, DPSC are also able to readily form neurospheres. The therapeutic effect of DPSC, however, is mostly considered through a paracrine mode of action, which entails various trophic and anti-inflammatoryary factors. Because of the aforementioned neurogenic and neurotrophic properties, DPSC have a tremendous impact on neuroregenerative therapy (Ovindasamy et al., 2010; Mead et al., 2017; Mohan and Ramalingam, 2020).

Previous studies examining co-cultures of molar pulp explants with trigeminal ganglia (TG) from neonatal rat pups, demonstrated distinct neurite outgrowth suggesting that tooth pulp tissue secreted neurotrophic neurite-promoting factors to attract axons from local nerve trunks in TG/pulpal co-cultures (Lillesaar et al., 1999).

**Mechanism of action of DPSC in the repair of neural elements:**
Studies from different laboratories have highlighted that paracrine mechanism is responsible for the improved neuronal function rather than neuronal differentiation from the stem cells (Mead et al., 2013, 2017; Mohan and Ramalingam, 2020). Mead et al. compared the neurotrophic activities of human DPSC, human bone marrow-derived MSC and human adipose-derived stem cells on axotomised adult rat retinal ganglion cells in vitro and concluded that human DPSC promoted significant multi-factorial paracrine-mediated retinal ganglion cells survival and neurite outgrowth and may be considered a potent and advantageous cell therapy for retinal nerve repair (Mead et al., 2014).

We have previously explored the application of DPSC-derived trophic factors to mediate axonal guidance and neuronal survival in TG neurons which was due to the presence of neurotrophic factors (NTFs) in the collected media from DPSC cultures (DPSC-CM/conditioned media). CM was collected from sub-confluent DPSC that were cultured in serum free media for 72 hours. Specific enzyme-linked immune sorbent assays were used to detect the secreted NTFs including NGF, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF) and neurotrophin-3 (NT-3). Quantitative polymerase chain reaction qPCR confirmed upregulation of neuronal markers in TG neuronal cultures underscoring that DPSC-derived NTFs upregulated genes responsible for neuronal survival and neurite outgrowth extension (Sultan et al., 2020).

To explore the role of DPSC-secreted NTFs on neuronal protection and neurite outgrowth extension further, specific neutralizing antibodies against GDNF and NT-3 were used to block the biological activity of the corresponding NTFs in DPSC-CM (Figure 1). The neutralizing experiments were conducted to determine the exact role of NTFs detected in DPSC-CM on neuronal survival and neurite outgrowth by adding neutralizing antibodies against; GDNF and NT-3 to the DPSC-CM before adding it to the cell culture. To further validate that these NTFs were totally knocked out, enzyme-linked immune sorbent assays analysis was assessed on the CM after adding the neutralizing antibodies. It was found that adding 5 µL/mL GDNF antibody and 10 µL/mL NT-3 antibody totally blocked the corresponding NTFs level in the DPSC-CM.

**Morphometric analysis of immunocytochemically stained images using ImageJ software (National Institutes of Health, Bethesda, MD, USA).** Lillesaar et al. (1999) revealed that neutralization of GDNF in DPSC-CM treated primary TGNC cultures resulted in only a slight reduction of the number of surviving neurons as assessed by NeuN immune marker, but significantly attenuated the neurite outgrowth as assessed by MAP-2 immune marker (Figure 1). This result suggested that GDNF was particularly important for TG neurite outgrowth promoted by DPSC-derived factors. Cellular processes influenced by GDNF such as proliferation, differentiation, maturation and neurite outgrowth of GDNF have been recognized but seem to have been less studied than its neurosurvival promoting effect. Our finding that inhibiting GDNF resulted in effects on both TG neuronal survival and neurite outgrowth underlined a critical dual function of this NTF.

Interestingly, blocking of NGF by the neutralizing anti-NGF antibody did not significantly affect both neuronal survival and neurite outgrowth in TG cultures (data not shown). The cultured neurons were still able to extend processes even after the addition of neutralizing antibody against NGF. These results were in accordance with Lillesaar et al. (1999) who demonstrated that NGF antibody failed to inhibit TG neurite growth promoted by DPSC suggesting that unidentified factor(s) induce neurite outgrowth in vitro. While we previously confirmed that neutralization of NGF in DPSC-CM was critical for PC-12 neurite outgrowth (Sultan et al., 2021).
At the same time, neutralization of NT-3 did not significantly affect TG neurite length; however, it did significantly reduce the number of viable TG neurons as assessed by immunocytochemical mature neuronal marker NeuN (Figure 1). Buchman and Davis (1993) suggested that NT-3 is a target factor for TG neuronal support as they observed abundant expression of NT-3 mRNA in TG culture. They also found that TG cell survival depends on different NTFs at different developmental stages with NT-3 mainly impacting neuronal cell survival. Noteworthy, our previously reported data showed that blocking of NT-3 did not result in any observed influence on DPSC-CM stimulated neuronal cell line (PC-12) survival or neurite outgrowth which means the possibility of having two different actions of the same neutralizing antibody on two different cell lines (Sultan et al., 2021).

In conclusion, the soluble secreted factors from DPSC promote neuronal cell survival and axonal regeneration and may be considered as cell-free therapy for nerve repair. The secreted NTs can be easily harvested, purified and stored, thus avoiding complications associated with cell therapy, such as unwanted proliferation/differentiation. Here we have provided further evidence of a critical role of GDNF and NT-3 in TG neuronal cell survival and regeneration. Further studies are warranted to identify the key DPSC-associated neural bioactive factors, to investigate the therapeutic efficiency of the DPSC secretome to support development of novel DPSC-based neuronal therapies.

Nessma Sultan*, Ben A. Scheven
Department of Oral Biology, Faculty of Dentistry, Mansoura University, Mansoua, Egypt (Sultan N)
School of Dentistry, Oral Biology, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK (Scheven BA)
*Correspondence to: Nessma Sultan, PhD candidate, nesmasultan88@gmail.com. https://orcid.org/0000-0003-9695-9170
(Nessma Sultan);
https://orcid.org/0000-0002-3219-3906
(Ben A. Scheven)

Date of submission: February 22, 2021
Date of decision: March 16, 2021
Date of acceptance: May 25, 2021
Date of web publication: August 30, 2021

https://doi.org/10.4103/1673-5374.322449

How to cite this article: Sultan N, Scheven BA (2022) Exploring the potential application of dental pulp stem cells in neuroregenerative medicine. Neural Regen Rev 17(4):775-776.

Copyright license agreement: The Copyright License Agreement has been signed by both authors before publication.

Plagiarism check: Checked twice by iThenticate.
Peer review: Externally peer reviewed.
Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

References
Buchman VL, Davies AM (1993) Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development 3:989-1001.

Liljeqvist C, Eriksson C, Johansson CS, Fried K, Hildebrand C (1999) Tooth pulp tissue promotes neurite outgrowth from rat trigeminal ganglia in vitro. J Neurocytol 8:663-670.

Mead B, Logan A, Berry M, Leadbeater W, Scheven BA (2013) Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. Invest Ophthalmol Vis Sci 54:7544-7556.

Mead B, Logan A, Berry M, Leadbeater W, Scheven BA (2014) 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 10: e109305.

Mehd B, Logan A, Berry M, Leadbeater W, Scheven BA (2017) Concise review: dental pulp stem cells: a novel cell therapy for retinal and central nervous system repair. Stem Cells 35:1-61-67.

Mohani SP, Ramalingam M (2020) Dental pulp stem cells in neuroregeneration. J Pharm Bio Allied Sci 12:60-66.

Ovdinasamy V, Abdullah AN, Ronald V5, Musa S, Ab Aziz ZA, Zain RB, Totey S, Bhonde RR, Abu Kasim NH (2010) Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. J Endod 36:1504-1515.

Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wakao N, Imagama S, Hibi K, Kadomatsu K, Ishiguro N, Ueda M (2012) Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 130:80-90.

Sultan, N, Amin, LE, Zaher AR. Grawish ME, Scheven BA (2020) Paracrine-mediated neuroprotection of dental pulp stem cells in trigeminal neural cells. Sci Rep 10:19694.

Sultan N, Amin LE, Zaher AR, Grawish ME, Scheven BA (2021) Dental pulp stem cells stimulate neuronal differentiation of PC12 cells. Neural Regen Rev 9:1821-1828.

Tay AB, Zuniga JR (2007) Clinical characteristics of trigeminal nerve injury referrals to a university centre. Int J Oral Maxillofac Surg 10:922-927.

Zheng WH, Quirion R (2004) Comparative signalling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival. J Neurochem 4: 844-852.