Dental Pulp Stem Cells in Neuroregeneration

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ABSTRACT

Neurological diseases and injuries affect the routine life of patients. Current medical and surgical treatment has not improved the quality of life to desired limits. Neural regeneration through stem cells may be ideal choice in current scenario. Dental pulp stem cells (DPSCs), which are isolated from dental pulp, have shown excellent neuroregenerative properties in various animal studies. This review outlines the clinical perspective of DPSCs in neuroregeneration.

KEYWORDS: Dental pulp stem cells, nerve regeneration, neuroregeneration

INTRODUCTION

Dental pulp is a vital structure present in the inner core of tooth containing mesenchymal stem cells (MSCs), fibrous tissue, neural fibers, blood components, vessels, and lymphatics. Unlike bone, teeth do not undergo continuous regeneration except for limited regeneration by forming reparative dentin through progenitor cells or stem cells from dental pulp.[1,2] Gronthos et al.[3] in 2000, isolated the stem cells from dental pulp and termed it as dental pulp stem cells (DPSCs). DPSCs are one of the widely researched tissues for its easy availability without morbidity to the human organs or tissues. It is considered as an organic waste, which can be retrieved easily from dental clinic trash bin. Teeth extracted for orthodontic purposes, and impacted third molars in young adult people form good source of stem cells.[4] Stem cells from exfoliated deciduous teeth (SHED) were isolated by Miura et al.[5] in 2003, and found that it has higher proliferation rate, population doublings, and better lineage than DPSCs. Various studies have proved DPSCs and SHED can differentiate into osteogenic, neurogenic myogenic, vasculogenic, chondrogenic, and adipogenic lineages.[6,7] Apart from these, dental stem cells can be isolated from apical papilla termed SCAP (stem cells from apical papilla) and periodontal ligament cells called PDLSC.
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Neuroregeneration is an exciting field, which is gaining attention due to the prevalence of various traumatic disorders, such as peripheral nerve injury, spinal cord injury, stroke, and also degenerative disorders such as Alzheimer’s disease and Parkinson’s disease. Conventional treatment, medical, and surgical therapies have limited ability to regenerate neurons [Figure 1]. Novel therapies, such as stem cells, may play a key role in regenerative neurology.[10] Neural stem cells will be the natural and ideal choice for the application in neurological disorders as they can readily differentiate into many neural cells, such as neurons, oligodendrocytes, and Schwann cell neurons. Ironically neural stem cells are difficult to harvest from brain and its related central and peripheral nervous system.[11] DPSCs unlike other stem cells have distinction, that is, it is derived from neural crest cells and has better lineage into neural tissues than bone marrow-derived stem cells (BMSC).[12] Considering the aforementioned facts, this article reviews the role of DPSC and SHED in the regeneration of neurological tissues in pathological and neuronal injury subjects.

**Harvesting, Isolation and Characterization of Dental Pulp Stem Cells**

Tooth extraction should be carried out in a sterile environment. Dental surgeon should carefully analyze to rule out any infection in the area of extraction. Immediately after extraction, tooth is immersed in 70% ethanol. Tooth was sliced at cemento-enamel junction with diamond disc. Care should be taken to avoid overheating during slicing by using water spray.[13] The pulp is removed using a sterile barbed broach and is placed in DMEM (Dulbecco’s Modified eagle medium), sliced into three pieces of 1 mm, and then incubated with trypsin. Trypsin was inactivated by adding DMEM and fetal bovine serum (FBS). The cells were passed through cell strainer to get single cell population and then centrifuged. It was then seeded into flask containing FBS and phosphate-buffered saline. Cells were kept in carbon dioxide incubator, and the medium was changed 24 h after seeding. In the gap of 3 days, the cells were passaged repeatedly until 80% confluence using trypsin. The cultured DPSCs were passed through markers such as STRO-1, CD44, CD105, CD29, CD90, CD271, and CD166. The cultured DPSCs were sorted through flow cytometry, and compulsory endotoxin test was conducted to rule out pathogens.[3] The DPSCs were tested for any genetic changes using karyotyping and PCR (polymerase chain technique). Alternative and advanced techniques of isolation and culturing were also carried out by researchers such as using serum-free media to avoid animal products in the culture. The type of tooth used for isolation also plays major role in the quality and quantity of DPSCs. The young tooth has

![Figure 1: Current clinical status in the management of neurological disorders](image_url)
more DPSCs than aged one; impacted third molar and tooth removed for orthodontic purposes having open apex have good number of DPSCs.

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DPSCs can be differentiated into neural elements when they are treated with neurogenic supplements such as epidermal growth factors (EGF), retinoic acid, fibroblastic growth factor (FGF) [Figure 2].[14] The low availability of neural stem cells for regeneration opens door for other stem cell such as BMSC and adipose stem cells (ASC). But, DPSCs have proved better alternative than other stem cells as it has intrinsic neural factors and also it is derived from neural crest. With this inherent capacity, DPSC will be able to readily form neurospheres.[15] The therapeutic effect of MSCs, especially DPSCs is through paracrine effect, which also secrete anti-inflammatory factors and trophic factors. The downside of DPSCs is through paracrine effect, which also secrete anti-inflammatory factors and trophic factors. The downside of DPSCs is the volume of the tissue (10–100 µL), especially from SHED. It requires several passages to get the ideal number, which might reduce the potency of the DPSCs.[16] Some researchers are able to show that DPSCs can be cultured over the period of 6 months without morphological changes. The aforementioned study has given tremendous impetus that DPSCs can be cultured in high numbers, which can be readily used for regenerative therapies.[17] Researchers were able to achieve 95% success rate in the extraction, isolation, and culturing of DPSCs. In contrast, some researchers were able to generate only neural progenitor cells not functional neuronal cells with DPSCs. They hypothesize that the pulp-to-pulp differences in human dental pulp can be one of the factors that determine neuronal differentiation.[18]

**Microenvironment of Neurons and Their Regeneration**

Microenvironment plays a major role in neuronal survival and its regeneration. Neurons of the central nervous system form the functional cells, which have interconnecting neural networks through axons, transmitting information via synapses. Four types of glial cells were found in microenvironment: oligodendrocytes, astrocytes, NG2 glia, and microglia. Oligodendrocytes are supporting cells that form myelin sheath, whereas microglia, the defense cells, respond to injuries and other insults. These cells are impregnated in extracellular matrix (ECM), thereby forming cell-to-cell interaction and ECM-to-cell interaction, which is the key for functioning of a neuron.[19] The pathophysiology of central nervous system (CNS) injury, spinal cord injury, and stroke is quite different, but the cellular responses are almost the same. The cellular responses are mainly apoptosis, necrosis, inflammation, activation of glial cells, free radical increase, and accumulation of toxins, leading to axonal degeneration and finally glial scar formation.[20] Microglia plays double-edged sword role by activating inflammatory mediators, which might help in eliminating pathogens and debris, at the same time triggering neurotoxic factors, which downregulates neurotrophic factors (NTF) that help in the survival of neural stem cell. To create an ideal environment for neuroregeneration, some neuroprotective factors will be ideal such as decreasing glial scar, regulating behavior of microglia, and generating more oligodendrocytes to generate myelin.[11] It is imperative to suppress miogial invasion in chronic neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease. Minocycline has proved to inhibit microglial activation in experimental animals. Natural products can play a major role in controlling microglial population in chronic degenerative diseases of CNS.[21] ECM plays an important role in neurodegenerative diseases, repair of nerve during injury, and regulation of synapse.[22] It is mainly made up of proteoglycans, among them, chondroitin sulfate proteoglycans (CSPGs) and heparan sulfate proteoglycans (HSPGs) form the major part of nervous system. Proteoglycans, which is a combination of carbohydrate and protein, play a major role in CNS repair.[23] CSPGs are one of the widely researched proteoglycans, which plays a major role in neuronal regeneration.

**Therapeutic Applications of Dental Pulp Stem Cells in Neuroregeneration**

Central nervous system regeneration

The CNS comprises brain and spinal cord, which controls all sensory and motor functions of the body. It is mired by limited capacity for regeneration or repair. Regeneration of these tissues can be preferably achieved by neural stem cells. DPSC which is of neural crest origin have neural factors inherently and also proven to be one of the best resources for the regeneration of neuronal tissues.[24] DPSC has shown neuroprotective effect in degenerative disease such as Parkinson’s disease and Alzheimers disease. Intracerebral transplantation of human DPSC in a cerebral ischemia rodent model showed significant improvement in sensorimotor function.[25] DPSCs generate large amount of NTF mRNA compared with BMSC, which proves its inherent neurogenic capacity. DPSCs transplanted into cerebral infarct due to artery occlusion were able to show regeneration through astrocyte-like cells.[26] Improvement in functional behavior is achieved in animal model by transplanting DPSCs into the ventricles.
of the brain.\({}\textsuperscript{27}\) It also turned into neuron-like cell after transplantation into avian embryo mesencephalon.\({}\textsuperscript{14}\) Predifferentiated DPSC with retinoic acid, erythrocyte growth factor, and bFG2 generates neural elements when it is transplanted into the cerebrospinal fluid of animals with total brain injury. The migration of DPSC-derived cells was observed in various CNS regions expressing sodium/potassium currents.\({}\textsuperscript{26}\) This proves that DPSCs can migrate and regenerate CNS-injured site either by predifferentiation or by paracrine effect.
Spinal cord

Human DPSCs when transplanted into spinal cord injury site of the rat showed significant positive regeneration\textsuperscript{[12]} Functional recovery was observed in complete transected spinal cord of rat using DPSCs. These studies proved that DPSCs were able to generate large amount of neurotrophic factors\textsuperscript{[12]} Neuroregenerative factors such as NT, brain-derived neurotrophic factor (BDNF), neurotrophic-3 (NT-3), and nerve growth factor (NGF) help in regeneration of axons\textsuperscript{[28]} DPSCs were able to promote survival of endogenous neurons in spinal cord injury without promoting axon regeneration. DPSCs regenerate through paracrine mechanism, which in turn induces oligodendrocyte differentiation, thereby forming myelination and regeneration of axons\textsuperscript{[29]} Some nonpermissive environment with paucity of trophic factors, especially neurogenic growth factors and increase in inhibitory molecules of axon can be one of the reasons for inadequate regeneration\textsuperscript{[30]} The immunophenotype of mouse DPSCs showed cell types from both central and peripheral nervous system. The aforementioned fact was proved by the positive expression of markers such as GFAP and S-100, respectively. SHED help in the replacement of damaged oligodendrocytes in spinal code injury animal model. These data prove SHED and DPSC are ideal models for spinal code injury\textsuperscript{[12]} SHED showed good survival when engrafted in spinal code injury animal models. While comparing with embryonic stem cell, which has only one percentage survival in engraftment, SHED showed 30\% engraftment. Engrafted SHED showed differentiation into oligodendrocytes\textsuperscript{[31]} DPSCs and SHED help in the regeneration of functional neurons in vitro and are able to express voltage-gated sodium channels\textsuperscript{[14]} SHED has a unique property compared with any other stem cell in body that the neural regeneration is achieved by inhibiting CSPGs and myelin-associated glycoproteins (MAG) by paracrine mechanism.

Peripheral nerve regeneration

The damage to sensory and motor peripheral nerves was managed earlier by autografting, which causes adverse effects such as causalgia and altered sensation. Various medical and surgical techniques were used to regenerate nerves, which have mixed results. Parenteral and local drug administration has its own limitations. Parenteral drugs take long time to act, and local drugs have higher concentration when applied in the area of interest because of the fast metabolism of drugs. Sustained local drug release is one of the favorable options that help in treating them medically. It mainly focus on the delivery of neurotrophic growth factors\textsuperscript{[32]} Tacrolimus and electrical stimulation have showed significant improvement in the regeneration of nerve injury in critical cases where the accessibility of nerve is compromised\textsuperscript{[33]} Nerve regeneration using stem cells can play a paramount role in future. Neural stem cells are ideal for regenerating nerves but cannot be isolated from CNS as it may invite clinical complication and ethical constraints. DPSC is an ideal choice of regeneration of severed nerve during surgery and trauma. DPSC-derived trophic factors have shown to mediate axonal guidance in trigeminal nerve\textsuperscript{[16]} Various experiments have proved their neuroregenerative potential in growing both sensory and motor nerves in animal models. DPSC filled in poly lactic-co-glycolic acid (PLGA) tubes showed good axonal regeneration, in which PLGA tube acted as the nerve guide in vivo. The PLGA disintegrates over time and do not need secondary intervention to retrieve it\textsuperscript{[34]} Collagen tubes filled with DPSCs have shown good regeneration of peripheral nerves in pigs. This has given an excellent impetus and boon to conduct clinical trials in human models, as pigs almost resemble human physiology in response to neural injury\textsuperscript{[35]} DPSC contains large number of Schwann cells, which has a paramount role in peripheral nerve regeneration. Facial palsy, which affects quality and appearance of the individual and also present with asymmetry of face, was treated with complicated reconstructive surgeries. Surgeries and physiotherapy may take long time for the improvement of the facial features with guarded results. Facial nerve defects were functionally regenerated with collagen tabulation using DPSCs. The regenerative nerve fiber was electrophysiologically active, which shows an active nerve conduction. The conduction was lower with DPSC-engrafted nerves, which is attributed due to immature myelin sheath formation. It will take at least 13 weeks to regenerate facial nerve to make it more conductive with DPSC. The DPSC has an advantage, when compared with ASCs in nerve regeneration, that is, the latter has a tendency to form adipocytes in the regenerative area, which is considered harmful.

Conclusion

DPSCs and SHED have considerable advantage compared with other stem cells such as BMSCs and ASCs in neural regeneration. Further advanced research is needed to understand pathophysiology of neural regeneration, especially mechanism of action of stem cells and their intrinsic factors in the repair of neural elements. The major challenge is the clinical translation of DPSCs in human subjects as many research work was on animal models. In animals, the nerves were severed and the transplantation was done.
within the given framework of time, which is not true in real trauma or pathologies. Advanced computer-simulated, artificial intelligence–guided regeneration might help in computing the exact permutation and combination of the techniques. The microenvironment, cell cycle, local factors, and survival of animal varies with human subjects. The quality and quantity of the DPSCs also play a major role in the success of regeneration. Pulp being very tiny in size, the number of teeth to be harvested might need to be increased if the amounts of stem cells are less. In that situation, intentional removal of pulp followed by root canal treatment can help in achieving the prescribed number of stem cells. Scaffold and local microflora also play a role in the success of regeneration. Recently with the advent of three-dimensional bio-printing, the guides for nerve regeneration can be conveniently designed to achieve better results.

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Conflicts of interest
There are no conflicts of interest.

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