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The Effect of Biomaterials on Human Dental Pulp Stem Cell Neural Differentiation: A Scoping Review

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Abstract

Neural cells are the most important components of the nervous system and have the duty of electrical signal transmission. Damage to these cells can lead to neurological disorders. Scientists have discovered different methods, such as stem cell therapy, to heal or regenerate damaged neural cells. Dental stem cells are among the different cells used in this method. This review attempts to evaluate the effect of biomaterials mentioned in the cited papers on differentiation of human dental pulp stem cells (hDPSCs) into neural cells for use in stem cell therapy of neurological disorders. We searched international databases for articles about the effect of biomaterials on neuronal differentiation of hDPSCs. The relevant articles were screened by title, abstract, and full text, followed by selection and data extraction. Totally, we identified 731 articles and chose 18 for inclusion in the study. A total of four studies employed polymeric scaffolds, four assessed chitosan scaffolds (CS), two utilised hydrogel scaffolds, one investigation utilised decellularised extracellular matrix (ECM), and six studies applied the floating sphere technique. hDPSCs could heal nerve damage in regenerative medicine. In the third iteration of nerve conduits, scaffolds, stem cells, regulated growth factor release, and ECM proteins restore major nerve damage. hDPSCs must differentiate into neural cells or neuron-like cells to regenerate nerves. Plastic-adherent cultures, floating dentosphere cultures, CS, polymeric scaffolds, hydrogels, and ECM mimics have been used to differentiate hDPSCs. According to our findings, the floating dentosphere technique and 3D-PLAS are currently the two best techniques since they result in neuroprogenitor cells, which are the starting point of differentiation and they can turn into any desired neural cell.

Keywords: Biomaterials, Human Dental Pulp Stem Cell, Neural Differentiation

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Introduction

The scientific community has placed considerable emphasis on the investigation of stem cells and their therapeutic applications. The pluripotent and multipotent characteristics of stem cells render them indispensable in tissue engineering methodologies, as they facilitate tissue regeneration and repair (1). Dental pulp tissue, which is developed from the neural crest, can now be acquired, separated, and conserved for extended periods of time through non-invasive and innovative techniques, such as retrieval from extracted wisdom teeth and shed deciduous teeth (2). Mesenchymal stem cells, which are a type of stem cells that exist in bone marrow in limited quantities, exhibit mesodermal tissue differentiation and are capable of differentiating into all three germ layers (3, 4). Multipotent stem cells, known as human dental pulp stem cells (hDPSCs), can be readily extracted from pulp tissue and have the capability to differentiate into diverse cell types, including adipocytes, osteocytes, glial cells, and neural cells because of their heterogeneous character (5). The aforementioned characteristic renders them a non-invasive and ethically sanctioned source of mesenchymal-like stem cells. hDPSCs that originate from neural crest exhibit neuro-ectodermal specification (5, 6). This allows them to generate different neurotrophic factors, like nerve growth factor (NGF) and glial cell line-derived neurotrophic factor, which confer neuroprotective properties (7, 8). It has been shown that hDPSCs express neural markers when transplanted into a rodent brain (9). The results of other

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studies demonstrated the differentiation of these hDPSCs into specific kinds of neural cells, including spiral ganglion neuron and inner ear hair cells (10, 11).

Neural cells are fundamental components of the nervous system that enable the propagation of electrical signals. As a result, harm or impairment to these cells can lead to diverse neurological conditions that are contingent on the specific type of neuron affected (2). Various disorders can arise due to impairment in either the central nervous system or peripheral nervous system, including Parkinson's disease, spinal cord injuries, and Alzheimer's disease, among others (12, 13). The treatment of neurodegenerative disorders is a complex process owing to the restricted self-repairing ability of damaged neural cells and their incapacity to regenerate lost cells. Cell-transplant therapy has been investigated as a means to augment endogenous nerve regeneration. hDPSCs present a potential avenue for the restoration of neuronal damage through the application of tissue engineering methodologies, which is due to their capacity to undergo neuronal differentiation and endure harmful conditions associated with lesions (14). Nonetheless, a significant obstacle pertains to the proficient administration of these cells to the intended location and the establishment of a biocompatible milieu for cellular differentiation. The identification of an appropriate biomaterial for the transfer of cells and facilitation of their differentiation into specific cell types is a critical aspect of cell therapy (12, 15).

The integration of scaffolds, cells, and growth factors is a fundamental aspect in the development of structures utilised in tissue engineering (13). Scaffolds have an important effect on cell maintenance, growth, and differentiation, as well as in facilitating cell migration and adhesion by providing a suitable environment. Consequently, it is imperative that they exhibit distinct characteristics, including elevated biocompatibility, biodegradability, absence of toxicity, and lack of inflammatory properties. Scaffolds frequently employed in tissue engineering include chitosan scaffolds (CS), fibrin, collagen, fibronectin, polylactic acid scaffolds (3DP-PLAS), and natural or synthetic silk. Consideration of factors that include, but are not limited to pore size, texture, and mechanical strength, are imperative in the evaluation of these scaffolds. The structures in question are subject to two primary concerns, namely their limited cell-holding capacity and inadequate extracellular matrix (ECM) (7, 14, 16, 17). To mitigate these limitations, a plausible approach involves the incorporation of exogenous biomaterials and the application of biomolecules and proteins to the scaffold. This strategy enhances the scaffold's functionality by creating a more conducive environment that offers ample material and surface area for stem cell proliferation and differentiation. An alternative approach involves the development of an ECM-like architecture that utilises hydrogels or decellularised ECM, which can also provide the necessary substrate and surface (3, 8, 16).

The crucial significance of neural cells lies in their function of propagating electrical impulses throughout the nervous system. Consequently, any aberration in the functioning of these cells may lead to a disrupted nervous system. Hence, it is evident that a secure and ethically sanctioned approach for the regeneration of neural cells and nerves is imperative. hDPSCs represent a promising avenue for tissue engineering in the context of neuronal injury and neurodegenerative conditions, given their non-invasive acquisition. Therefore, it is imperative to ensure the optimal utilisation and cultivation of these cells. The objective of this investigation is to gather and present scholarly inquiry centred on the development of settings that can proficiently transfer these cells to the designated location and direct their differentiation into the targeted neuronal cells. Since neuro regenerative treatments are a current research concern and are one of the main problems of today's medicine, this study could create a new viewpoint on healing neurodegenerative diseases and their treatments.

Materials and Methods

This scoping review was conducted by following the PRISMA guidelines.

Search strategy and selection

The objective of this scoping review was to identify and assess research studies that centred on the neural differentiation of dental stem cells. We identified 18 studies that were published between 2014 to 2023 after a comprehensive search of the PubMed, Scopus, and Web of Science databases utilising the search flowchart outlined in Figure 1. The reviewed studies were all English. There were no restrictions placed on the search. The search methodology involved manual execution, and all pertinent studies were identified and subjected to a comprehensive review. The inquiry utilised four principal keywords, namely "neural", "differentiation", "dental", and "stem cells", alongside associated keywords derived from MeSH terms. EndNote software was utilised for the purpose of managing the studies and references that were searched. These studies and references were evaluated on the basis of their titles and abstracts. Redundant and non-related research works were identified and eliminated. The identification of duplicate entries was conducted by examining the titles of previously published articles, the year of publication, and the authors. Research relevant to the subject matter was thoroughly scrutinised, and only the outcomes that were relevant to the subject were chosen. Studies related to the topic were independently evaluated and selected by two researchers (MK and DA). The elimination process, which involved exclusion and inclusion criteria, was verified for accuracy by one researcher (RRD).

Exclusion and inclusion criteria

Any study that investigated the neural differentiation of dental stem cells was included in this scoping review. Duplicate citations, studies for which the full text was not available, animal studies, and unrelated outcomes were excluded.

Data collection and extraction

The objective of this scoping review was to identify

and assess research studies related to the topic of this study. The data acquired from the search were input into EndNote software, and an initial compilation of the necessary information was carried out by two researchers (MK and DA) through a review of the titles and abstracts of the articles. We screened 731 English articles published from 1968 to 2023. Animal studies were excluded from the analysis. After screening and finding the studies related to this scoping review, the primary author (MK) examined the full text of 43 studies and chose 18 that had the necessary information for conducting this research.

The data collection was based several key factors: authors and publication year, cell source, study type, biomaterial utilised, impact on differentiation, differentiation review technique, neural expression marker assessment, and resultant neural cell type.

Quality of studies

The first author (MK) assessed the studies and reported the required data according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

Primary and secondary endpoints

We primarily aimed to evaluate all available literature to identify and introduce the best biomaterial available to differentiate hDPSCs into neural cells based on the data provided by these papers. Our second aim was to evaluate and introduce cultures and growth factors used in this process that were published in these relevant papers.

Results

In the preliminary investigation, we detected 731 articles in the three databases: PubMed (n=151), Web of Science (n=132), and Scopus (n=448). From these, 290 duplicate papers were detected through automated means and 237 were identified manually. Upon conducting an evaluation of 168 studies based on their titles and abstracts, a total of 43 references were selected for further assessment of their full texts. In the end, a total of 18 articles were deemed eligible for inclusion and subsequent data extraction. Figure 1 contains all of the extracted data. Exclusion criteria were applied to papers that incorporated stem cells obtained from individuals with medical conditions. One article was found to be inaccessible and subsequently excluded.

The biomaterial most commonly utilised in the reviewed literature was the "chitosan porous scaffold", which was featured in a total of four articles. Four studies employed polymeric scaffolds, while two used hydrogel scaffolds. One investigation utilised decellularised ECM, and six studies applied the floating sphere technique during the differentiation process. An article was conducted to compare three protocols for the differentiation of hDPSCs into cholinergic neural cells. All techniques and materials employed were efficacious in generating neural cells or neuron-like cells, and demonstrated at least one positive neuronal expression marker assay.

The extracted data and obtained results are expected to be of tremendous value to researchers globally who are engaged in the exploration of neuron regeneration therapy and cell engineering. We noted advantages and disadvantages for each biomaterial. hDPSCs appear to have the potential to turn into neural cells; however, the resultant cells depend on the techniques and materials used in the media and differentiation process (Table 1).

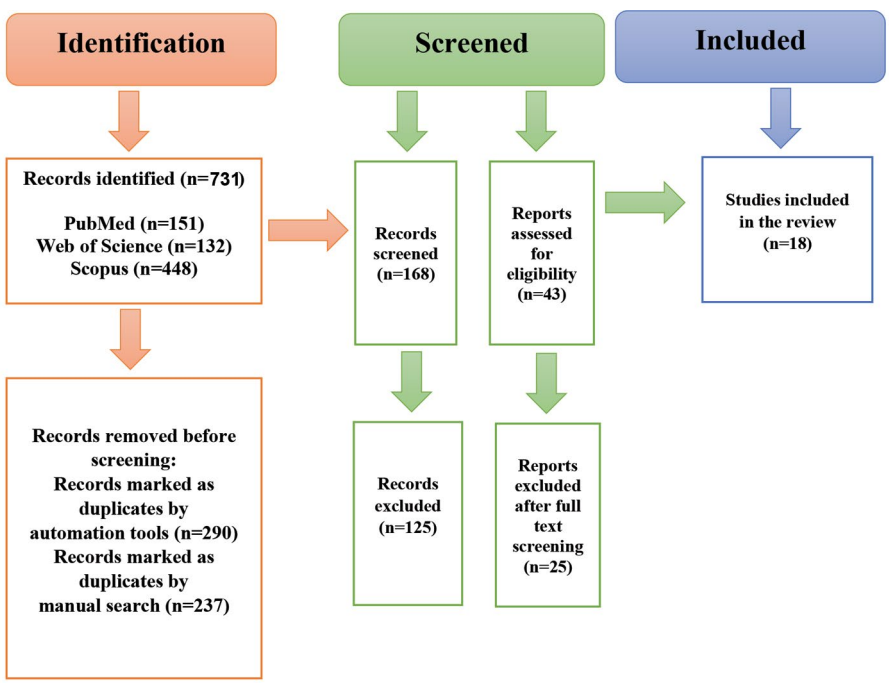

Fig.1: Identification of studies via databases and registers.

Table 1: The effect of biomaterials on hDPSC neural differentiation

Reference	Authors	Year of publication	Cell source	Type of study	Biomaterial		Impact on differentiation	Differentiation review technique	Neuronal expression marker check	Resultant neuron type
					Scaffold	Growth factors/ cultures				
(14)	Pineda et al.	2022	hDPSC	*In vitro*	Nanopatterned poly(lactide-co-caprolactone) (PLCL) scaffolds	Plastic-adherent monolayer cultures, floating dentosphere cultures	Indirect	ICC Flow Cytometry	Neural stem cell marker Nestin, glial markers GFAP, and S100ß, and neuronal markers [NeuN and doublecortin (DCX)]	Not mentioned
(3)	Gao et al.	2022	hDPSC	*In vitro*	TPS-PN plates (poly-N-isopropylacrylamide-co-butyl acrylate coated TPS plates)	rVT, LN, PLO, PBS, PS, DPBS, Trypsin-EDTA, α-MEM, FBS, neurobasal-A, bFGF, EGF, B27, PN	Indirect	Flow cytometry, immunostaining	βIII-tubulin, nestin	Not mentioned
(18)	Drewry et al.	2022	Excluded (due to not being accessible)	---------	----------	----------	----------	----------	----------	----------
(7)	Zheng et al.	2021	hDPSC	*In vitro*	Chitosan porous scaffolds	Low glucose DMEM, FBS, penicillin, streptomycin	Indirect	Western blot, IF staining, RT-PCR	GFAP, S100β, β-tubulin III	Not mentioned
(16)	Luo et al.	2021	hDPSC	*In vitro*/ in vivo Sprague-Dawley male rats	GelMA-bFGF hydrogels		Indirect	Immunohistochemical analysis, Western blot	GFAP, β-tubulin III	Not mentioned
(19)	Farhang et al.	2021	hDPSC	*In vitro*	3D hanging drop technique	Neurobasal media and 10% foetal calf serum containing 20 ng/ml EGF, 40 ng/ml human bFGF (hbFGF), SHH protein	Indirect	ICC	β-tubulin III and NeuN	Not mentioned
(8)	Laudani et al.	2020	hDPSC	*In vitro*	Decellularized ECM scaffold	FBS, penicillin, streptomycin, and fungizone, trypsin/EDTA, a-MEM, ascorbic acid	Indirect	Cytofluorimetric, IF, SEM, RT-PCR	MAP2, β-III tubulin, neurofilament-heavy (NF-H), VIM, NF-L, PAX6	Not mentioned
(17)	Hsiao et al.	2020	hDPSC	*In vitro*	3DP-PLAS	----------	Indirect	IF staining	GFAP, nestin, neurofilament-M (NF-M), β-III tubulin, MAP2	Neural precursor cells, neurogenic structures, astrocyte-like cells

Table 1: Continued

Reference	Authors	Year of publication	Cell source	Type of study	Biomaterial		Impact on differentiation	Differentiation review technique	Neuronal expression marker check	Resultant neuron type
					Scaffold	Growth factors/ cultures				
(20)	Rafiee et al.	2020	hDPSC	*In vitro*	Neurosphere technique	20 ng/ml EGF, 20 ng/ml bFGF, and 10 mg/ml heparin/ high-glucose DMEM with 10% FBS	Indirect	Quantitative polymerase chain reaction (qPCR), Western blot, and IF, Immunostaining, Western blot	MAP2, and neurogenin 1 (Ngn1)	Neural progenitor cells
(21)	Goudarzi et al.	2020	hDPSC	*In vitro*	Neurosphere technique	5% E-CSF	Indirect	ICC, RT-PCR	Nestin, MAP2, Oct4, Sox2, NF-M	Not mentioned
(2)	Kang et al.	2019	hDPSC	*In vitro*	----------	Protocol 1: BME and nerve growth factor (NGF) Protocol 2: D609 Protocol 3: bFGF, SHH, and RA	Indirect	Flow cytometry, cell cycle analyses, RT-qPCR, ICC, analysis of acetylcholine (Ach) secretion in culture media	ChAT, HB9, ISL1, BETA-3, MAP2	Cholinergic neuronal-like cells
(5)	Pisciotta et al.	2018	hDPSC	*In vitro*	3D floating sphere culture system	EGF, bFGF, DMEM/F12 culture medium, L-glutamine penicillin, streptomycin, B27	Indirect	IF analysis, Western blot, pseudocolour analysis	Nestin, CD271, SOX-10, β-III tubulin, MAPٲ	Neural progenitor cells, neural crest stem cells
(6)	Bojnordi et al.	2018	hDPSC	*In vitro*	Neurosphere technique	bFGF, EGF, B27, N2, poly-L-lysine-coated coverslips	Indirect	Flow cytometry, IF staining	Nestin, NF68, MAP2, β-tubulin	Neuroprogenitor cells, mature neural cells
(12)	Ghasemi Hamidabadi et al.	2017	hDPSC	*In vitro*	Chitosan-intercalated montmorillonite/PVA nanofibers	Serum-free DMEM/F12 with 2% B27 supplemented with 20 ng/mL bFGF and 20 ng/mL EGF	Indirect	RT-PCR, immunostaining	Oct-4, Nestin, NF-M, NF-H, MAP2, β-III tubulin	Neuron-like cells
(22)	Geng et al.	2017	hDPSC	*In vitro*	----------	Resveratrol, DMEM/F12, 10 ng/ml bFGF, 500 μM BME and 10% FBS	Indirect	ICC staining, Western blot, RT-PCR	Nestin, Musashi and NF-M	Neuroprogenitor cells
(13)	Zhang et al.	2016	hDPSC	*In vitro*	Chitosan porous scaffolds	DMEM, FBS, penicillin, streptomycin	Indirect	RT-PCR, Western blot, IF assays	CNPase, MAP2, GFAP	Oligodendrocytes, astrocytes, neural cells

Table 1: Continued

Reference	Authors	Year of publication	Cell source	Type of study	Biomaterial		Impact on differentiation	Differentiation review technique	Neuronal expression marker check	Resultant neuron type
					Scaffold	Growth factors/ cultures				
(23)	Martens et al.	2014	hDPSC	*In vitro*	Collagen hydrogel	Cell suspension, MEM, type I rat tail, sodium hydroxide/ alpha modification (alpha-MEM) FBS, glutamine, penicillin, streptomycin, BME, trans-RA, forskolin, bFGF, platelet-derived growth factor AA (PDGFaa), heregulin-beta-1 (NRG)	Indirect	ICC, ELISA	laminin, p75, GFAP, CD104, nestin	Schwann cell-like phenotype
(15)	Feng et al.	2014	hDPSC	*In vitro*	3D highly porous CS	Neural induction medium (NIM)-DMEM/F-12 medium, B27, N2, BDNF, NGF, bFGF	Indirect	RT-PCR, Western blot, IF	Nestin, CNPase, MAP2, GFAP	MAP-2+ neural cells, GFAP+ astrocytes and CNP+ oligodendrocytes

hDPSC; Human dental pulp stem cell, FBS; Foetal bovine serum, DMEM; Dulbecco's Modified Eagle medium, bFGF; Basic fibroblast growth factor, EGF; Epidermal growth factor, BME; β-mercaptoethanol, SHH; Sonic hedgehog, RA; Trans-retinoic acid, RT-PCR; Reverse transcription-polymerase chain reaction, IF; Immunofluorescence, ICC; Immunocytochemistry, GFAP; Glial fibrillary acidic protein, MAP2; Microtubule-associated protein 2, CS; Chitosan scaffold, PVA; Poly (vinyl alcohol), and D609; Tricyclodecane-9-yl-xanthogenate.

Discussion

The third stage of nerve conduit development for repairing nerve injuries involves the integration of scaffolds, stem cells, controlled release of growth factors, and ECM proteins. This represents a more advanced iteration of the second generation. According to Luo et al. (16), this particular method has demonstrated efficacy for restoration of substantial nerve damage. Additional research is required to establish the effectiveness and safety of utilising hDPSCs as the stem cell constituent in this approach. Notably, Yang et al. (24) assessed the capability of three different stem cell groups gathered from teeth-dental follicle stem cells (DFSCs), stem cells from apical papilla (SCAPs), and DPSCs to repair a spinal cord injury in rats. Although most dental stem cells secreted nestin and β-III tubulin, interestingly, the DFSCs had better proliferation potential and this indicated their ability to adapt to the environment. It is necessary to determine successful techniques for their proliferation and differentiation into specific cell lineages (4).

According to the literature, hDPSCs have the potential to differentiate in two distinct environments, namely plastic-adherent cultures and floating dentosphere cultures (14). The scaffolds employed in this process of differentiation are subject to modification (13). Scaffolds are utilised as a microenvironment that emulates the function of the ECM in cellular growth and differentiation (15).

CS are frequently utilised in conjunction with diverse growth factors and ECM proteins in these methodologies. The aforementioned scaffold possesses porous properties and can be manipulated into various shapes to facilitate its application in hDPSC neural differentiation. Additionally, it serves as a vehicle for conveying differentiated cells to the site of injury. It has been observed that the combination of CS with hDPSCs leads to an elevated probability of neural differentiation. The crucial attributes of CS are the means pore diameter and elevated swelling efficacy. A pore size of 268.79 ± 13.25 μm is deemed appropriate for neural growth and migration (7, 12, 13, 15).

Research conducted by Zheng et al. (7) involved the integration of CS with basic fibroblast growth factor (bFGF) and ERK/p-ERK to enhance neural differentiation of hDPSCs. bFGF is a biological factor that possesses a brief half-life and necessitates a stable milieu for the purpose of directing the differentiation of stem cells. Nonetheless, the primary limitations of chitosan are its inadequate hydrophilicity and suboptimal thermal and physiochemical characteristics. Ghasemi Hamidabadi et al. (12) have tackled the aforementioned concerns through the amalgamation of organic monmorillonite (OMMT) and chitosan to enhance the physical and thermal characteristics. Additionally, they have incorporated poly (vinyl alcohol) (PVA) to augment hydrophilicity. The results revealed that an increased concentration of OMMT (5%) resulted in a greater induction of neural differentiation. Also, another use of chitosan was explored by Mu et al. (25). They assessed the effect of chitosan tubes immersed with DPSCs and stem cell factor (SCF) on facial nerve-vascularised regeneration in rabbits. They reported that the combination of SCF and DPSCs had a positive effect on DPSC migration, activity and proliferation. SCF also provoked DPSCs neural differentiation. The SCF+DPSCs mixture inoculated in chitosan tubes prompted axonal regeneration and remyelination, and restored the function of regenerated nerve.

Polymeric scaffolds represent an alternative approach for the delivery and support of hDPSCs. PLAS are scaffolds that have been used for adhesion of hDPSCs, and the rate of their maturation is influenced by the dimensions of the pores present in these scaffolds. Pores of significant size are conducive to nourishment; although smaller pores offer a greater surface area, they may lead to necrosis. Scaffolds created with emerging technologies, such as 3D printing, have been explored. The width of the gap between struts has been identified as a crucial element in this process (17). Reducing the width of these gaps to 150 μm promotes cellular alignment, and application of poly-L-lysine or alcohol immersion enhances cell adhesion on these structures. Control of the sizes of the pores and gaps provides tremendous advantage for polymeric scaffolds.

Investigation of functionalised nanopatterned PLCL scaffolds showed their ability to enhance the requisite environment for cellular differentiation and migration. The significance of time and temperature are crucial variables during scaffold fabrication and should be duly acknowledged. Various techniques for surface functionalization have been employed to improve cell adhesion, such as plasma and polydopamine (PDA) treatment, graphene oxide (GO), and poly-D-lysine coating. Nonetheless, these techniques may impact the nanotopography of the initial film, and could potentially diminish the surface patterning effect by impeding direct interaction between stem cells and the scaffold surface (14). The ability of resveratrol, a polyphenol, to promote neural differentiation of hDPSCs was also investigated. The results indicated that incubation of cells in 15 μM resveratrol for 12 hours provided the optimum condition for successful differentiation (22).

Hydrogels are considered a viable approach for creating the necessary microenvironment for growth and viability of the hDPSCs. According to the results by Luo et al. (16), GelMA hydrogels that incorporated 10% bFGF exhibited superior biocompatibility, degradability, pore size, and swellability. The physical characteristics of the hydrogel, such as its pore structure, cross-linking, and overall polymer content have an impact on its properties. These properties, in turn, impact the circulation of nutrients and oxygen in addition to the release of waste materials. The introduction of bFGF into the scaffold facilitates the proliferation and spread of hDPSCs. Another advantage of this biomaterial is its solubility compared to CS.

Martens et al. (23) fabricated a specialised collagen gel using type I collagen extracted from rat tails, which effectively prompted the differentiation of hDPSCs into Schwann cells. The authors generated an engineered neural tissue that facilitated cellular alignment and guidance through the use of collagen, which ultimately promoted the cells to differentiate into Schwann cells.

One potential strategy for achieving hDPSC specialisation is through the replication of the ECM structure. According to Laudani et al. (8), the use of decellularised ECM obtained from stem cells in bone marrow is a viable option for scaffold material in this context. Their study revealed that the morphology and neural marker expression of the resultant cells were favourable and suggestive of differentiated cells with characteristics similar to neural cells. They emphasized that cellular differentiation and adhesion were augmented in this milieu when compared to a glass milieu.

Floating spheres is an alternative method to differentiate hDPSCs into the cells of interest. Conventional adherent techniques potentially disrupt the biological characteristics of cells. However, the 3D floating spheres have been shown to aid in the preservation of fibroblast-like morphology and embryological factors, while simultaneously maintaining differentiation and proliferation capabilities. These spheres are effective in helping hDPSCs differentiate into neural progenitor cells (5, 20). Bojnordi et al. (6) employed the neurosphere methodology in conjunction with poly-L-lysine coverslips to generate fully developed neural cells upon exposure to neural inducers. The neurosphere technique enhances neural crest stem cell marker expression of hDPSCs. Solis-Castro et al. (26) cultured hDPSCs in three different mediums with the sphere technique. They reported that BMP4 has a positive effect in neural marker expression when added to foetal bovine serum (FBS) and OSCFM cultures with this technique. The hanging drop technique is another 3D method. Farhang et al. (19) compared the 3D method with a simple 2D culture and observed that hDPSCs cultured by the 3D hanging drop technique enriched with Sonic hedgehog (SHH) and hbFGF had significantly higher potential for differentiating into cells that express neural markers. Spinal cord fluid is also

effective when combined with the dentosphere process and is another valid method for neural differentiation of hDPSCs (21).

Advanced Dulbecco's Modified Eagle Medium (ADMEM) is a commonly utilised culture medium for the growth and proliferation of hDPSCs. Alterations to this medium have the potential to induce neural differentiation in these cells. A study conducted by Kang et al. (2) compared three distinct protocols for the differentiation of cholinergic neural cells. Protocol I used β-mercaptoethanol (BME) and NGF. Protocol II incorporated tricyclodecane-9-yl-xanthogenate (D609) into the medium, and protocol III combined bFGF, SHH, and retinoic acid (RA) with the medium. The outcomes of all three protocols were comparable; however, protocol III demonstrated a marginally greater secretion of neurotransmitters and elevated expressions of neural markers, which was attributed to the combination of bFGF, SHH, and RA.

A comparative study assessed the efficacy of transplantation of hDPSCs and human dental pulp-derived induced neural cells (DP-iNCs) in the amelioration of functional recovery of mice and the results indicated that the DP-iNCs were more effective than DPSCs. DP-iNCs are better for the niche of the chosen site for transplantation and their ability in angiogenesis is superior to hDPSCs. Hence, hDPSCs appear to be better used in the laboratory for differentiation into the desired cells prior to transplantation (27). The results of studies showed that the additional neurotrophic factor was a pivotal aspect of the treatment of the injured nerve site. Another study showed that the addition of platelet rich plasma to the hDPSCs was effective due to the ability of PRP to provide nutrients, align the anti-inflammatory response, and prevent neuron cells from apoptosis (28).

In addition to cultures and scaffolds, other aspects of cell differentiation should be taken into account. For instance, DPSCs derived from the deciduous teeth of a Down Syndrome patient have a lower level of neural marker expression compared to the control group (29). Also, the cell cycle phase which the DPSC is in has an importance in the differentiation process (30).

Luo et al. (16) transplanted the resultant neural cells into rats. The laboratory results confirmed the success of this experiment. Since no specific research has been conducted directly on humans in this regard, more studies must be conducted to confirm the potential benefit of these stem cells in the clinical setting.

The most common media, growth factors, and cultures used in these studies were: bFGF in nine studies (2, 3, 12, 13, 15, 16, 19, 22, 26); penicillin in five studies (5, 8, 13, 16, 23); and FBS for generating media in five studies (7, 8, 13, 19, 23). The media was approximately the same in all mentioned studies; however, our main focus was scaffolds and we recommend additional studies be conducted to analyse the type of media for differentiating these stem cells into the targeted neural cells.

Diverse methodologies and substances have been investigated to establish ideal conditions for the differentiation of hDPSCs into fully functional neural cells or neuron-like cells. CS, polymeric scaffolds, hydrogels, and ECM mimicking strategies are all involved in enhancing the differentiation process. Our assessment of the cited articles indicates that hDPSCs have the potential to turn into neural cells. The important influencing factor is the technique used to obtain the desired cells. Continued exploration and improvements to these methodologies will further help nerve injury restoration and rejuvenation.

We recommend that more studies, including human clinical trials, should be conducted to confirm the feasibility of these cells in the clinic. It would be helpful to evaluate the media and culture used for differentiating hDPSCs into neural cells or neuron-like cells.

Conclusion

hDPSCs could be used in regenerative medicine to effectively treat nerve damage. Scaffolds, stem cells, regulated growth factor release, and ECM proteins restore major nerve damage. hDPSCs must differentiate into neural cells or neuron-like cells to regenerate nerves. Thus, plastic-adherent cultures, floating dentosphere cultures, CS, polymeric scaffolds, hydrogels, and ECM mimics have been used to differentiate hDPSCs. Studies show that pore size, swelling efficiency, scaffold composition, and growth factors affect hDPSC differentiation and survival. Therefore, due to these traits, many effective nerve injury healing methods have emerged. However, each has advantages and disadvantages. These methods should be improved to maximise nerve damage treatment and safety using hDPSCs. According to our findings, the floating dentosphere technique (e.g., the neurosphere technique) and 3D-PLAS appear to be the two best techniques currently available since they result in neuroprogenitor cells, which are the starting point of differentiation and they can turn into any of the desired neural cells. The most recent published work indicates that the focus is on improving nanopatterned scaffolds and assessing their roles in differentiation of hDPSCs. They are proven to be useful for this purpose.

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Authors' Contributions

Y.M.; Conceptualization and Methodology. Y.M., R.R.D., A.P.; Validation, Formal analysis, and Supervision. R.R.D.; Project administration, Data curation, and Writing – review and editing. M.K., D.A.; Investigation, Resources, Data curation, Writing – original draft and

Visualisations. R.R., S.A.S.; Validation and Investigation. All authors read and approved the final manuscript.

References

1. Biehl JK, Russell B. Introduction to stem cell therapy. J Cardiovasc Nurs. 2009; 24(2): 98-103.
2. Kang YH, Shivakumar SB, Son YB, Bharti D, Jang SJ, Heo KS, et al. Comparative analysis of three different protocols for cholinergic neuron differentiation in vitro using mesenchymal stem cells from human dental pulp. Anim Cells Syst (Seoul). 2019; 23(4): 275-287.
3. Gao Y, Tian Z, Liu Q, Wang T, Ban LK, Lee HH, et al. Neuronal cell differentiation of human dental pulp stem cells on synthetic polymeric surfaces coated with ecm proteins. Front Cell Dev Biol. 2022; 10: 893241.
4. Bongso A, Richards M. History and perspective of stem cell research. Best Pract Res Clin Obstet Gynaecol. 2004; 18(6): 827-842.
5. Pisciotta A, Bertoni L, Riccio M, Mapelli J, Bigiani A, La Noce M, et al. Use of a 3D floating sphere culture system to maintain the neural crest-related properties of human dental pulp stem cells. Front Physiol. 2018; 9: 547.
6. Bojnordi MN, Haratizadeh S, Darabi S, Hamidabadi HG. Neural derivation of human dental pulp stem cells via neurosphere technique. Bratisl Lek Listy. 2018; 119(9): 550-553.
7. Zheng K, Feng G, Zhang J, Xing J, Huang D, Lian M, et al. Basic fibroblast growth factor promotes human dental pulp stem cells cultured in 3D porous chitosan scaffolds to neural differentiation. Int J Neurosci. 2021; 131(7): 625-633.
8. Laudani S, La Cognata V, Iemmolo R, Bonaventura G, Villaggio G, Saccone S, et al. Effect of a bone marrow-derived extracellular matrix on cell adhesion and neural induction of dental pulp stem cells. Front Cell Dev Biol. 2020; 8: 100.
9. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells. 2008; 26(7): 1787-1795.
10. Adriztina I, Munir D, Sandra F, Ichwan M, Bashiruddin J, Putra IB, et al. Differentiation capacity of dental pulp stem cell into inner ear hair cell using an in vitro assay: a preliminary step toward treating sensorineural hearing loss. Eur Arch Otorhinolaryngol. 2022; 279(4): 1805-1812.
11. Gonmanee T, Thonabulsombat C, Vongsavan K, Sritanaudomchai H. Differentiation of stem cells from human deciduous and permanent teeth into spiral ganglion neuron-like cells. Arch Oral Biol. 2018; 88: 34-41.
12. Ghasemi Hamidabadi H, Rezvani Z, Nazm Bojnordi M, Shirinzadeh H, Seifalian AM, Joghataei MT, et al. Chitosan-intercalated montmorillonite/poly(vinyl alcohol) nanofibers as a platform to guide neuronlike differentiation of human dental pulp stem cells. ACS Appl Mater Interfaces. 2017; 9(13): 11392-11404.
13. Zhang J, Lu X, Feng G, Gu Z, Sun Y, Bao G, et al. Chitosan scaffolds induce human dental pulp stem cells to neural differentiation: potential roles for spinal cord injury therapy. Cell Tissue Res. 2016; 366(1): 129-142.
14. Pineda JR, Polo Y, Pardo-Rodríguez B, Luzuriaga J, Uribe-Etxebarria V, García-Gallastegui P, et al. In vitro preparation of human Dental Pulp Stem Cell grafts with biodegradable polymer scaffolds for nerve tissue engineering. Methods Cell Biol. 2022; 170: 147-167.
15. Feng X, Lu X, Huang D, Xing J, Feng G, Jin G, et al. 3D porous chitosan scaffolds suit survival and neural differentiation of dental pulp stem cells. Cell Mol Neurobiol. 2014; 34(6): 859-870.
16. Luo L, He Y, Jin L, Zhang Y, Guastaldi FP, Albashari AA, et al. Application of bioactive hydrogels combined with dental pulp stem cells for the repair of large gap peripheral nerve injuries. Bioact Mater. 2020; 6(3): 638-654.
17. Hsiao D, Hsu SH, Chen RS, Chen MH. Characterization of designed directional polylactic acid 3D scaffolds for neural differentiation of human dental pulp stem cells. J Formos Med Assoc. 2020; 119(1 Pt 2): 268-275.
18. Drewry MD, Dailey MT, Rothermund K, Backman C, Dahl KN, Syed-Picard FN. Promoting and orienting axon extension using scaffold-free dental pulp stem cell sheets. ACS Biomater Sci Eng. 2022; 8(2): 814-825.
19. Farhang S, Soleimani M, Ostadsharif M, Ghasemi N. Neurogenic induction of human dental pulp derived stem cells by hanging drop technique, basic fibroblast growth factor, and SHH factors. Dent Res J (Isfahan). 2021; 18: 57.
20. Rafiee F, Pourteymourfard-Tabrizi Z, Mahmoudian-Sani MR, Mehri-Ghahfarrokhi A, Soltani A, Hashemzadeh-Chaleshtori M, et al. Differentiation of dental pulp stem cells into neuron-like cells. Int J Neurosci. 2020; 130(2): 107-116.
21. Goudarzi G, Hamidabadi HG, Bojnordi MN, Hedayatpour A, Niapour A, Zahiri M, et al. Role of cerebrospinal fluid in differentiation of human dental pulp stem cells into neuron-like cells. Anat Cell Biol. 2020; 53(3): 292-300.
22. Geng YW, Zhang Z, Liu MY, Hu WP. Differentiation of human dental pulp stem cells into neuronal by resveratrol. Cell Biol Int. 2017; 41(12): 1391-1398.
23. Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, et al. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. FASEB J. 2014; 28(4): 1634-1643.
24. Yang C, Li X, Sun L, Guo W, Tian W. Potential of human dental stem cells in repairing the complete transection of rat spinal cord. J Neural Eng. 2017; 14(2): 026005
25. Mu X, Liu H, Yang S, Li Y, Xiang L, Hu M, et al. Chitosan tubes inoculated with dental pulp stem cells and stem cell factor enhance facial nerve-vascularized regeneration in rabbits. ACS Omega. 2022; 7(22): 18509-18520.
26. Solis-Castro OO, Boissonade FM, Rivolta MN. Establishment and neural differentiation of neural crest-derived stem cells from human dental pulp in serum-free conditions. Stem Cells Transl Med. 2020; 9(11): 1462-1476.
27. Matsumura H, Marushima A, Ishikawa H, Toyomura J, Ohyama A, Watanabe M, et al. Induced neural cells from human dental pulp ameliorate functional recovery in a murine model of cerebral infarction. Stem Cell Rev Rep. 2022; 18(2): 595-608.
28. Hu ZB, Chen HC, Wei B, Zhang ZM, Wu SK, Sun JC, 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(10): 584.
29. Pham TTM, Kato H, Yamaza H, Masuda K, Hirofuji Y, Sato H, et al. Altered development of dopaminergic neurons differentiated from stem cells from human exfoliated deciduous teeth of a patient with Down syndrome. BMC Neurol. 2018; 18(1): 132.
30. Gnanasegaran N, Govindasamy V, Kathirvaloo P, Musa S, Abu Kasim NH. Effects of cell cycle phases on the induction of dental pulp stem cells toward dopaminergic-like cells. J Tissue Eng Regen Med. 2018; 12(2): e881-e893.