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Review

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Are human dental pulp stem cells the future of neurodegenerative diseases and nerve injury therapy?*

Czy komórki macierzyste z miazgi zęba są przyszłością w leczeniu chorób neurodegeneracyjnych i uszkodzeń nerwów?

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Summary

This review summarizes results from *in vitro* and *in vivo* studies which provide evidence that human dental pulp stem cells (hDPSCs) might be a novel treatment strategy for nervous system injuries and neurodegenerative diseases because of their high potential for neurogenic differentiation and secretion of neuron-related trophic factors. It is also worth underlining that hDPSCs are neural crest-derived cells that possess biological properties of mesenchymal stem cells (MSCs). Induced hDPSCs have a high ability to differentiate into neuron-like cells, which show functional activity. hDPSCs express immunomodulatory factors that enhance regeneration and repair of nerve injury. These specific features of undifferentiated and differentiated hDPSCs make these cells promising for the therapy of neurodegenerative diseases, such as Alzheimer's, Parkinson's diseases, stroke, spinal cord injury as well as peripheral nerve injury. Recently, investigators propose that the tissue engineering technology, including scaffold, stem cells and growth factor, should provide a new strategy for spinal cord and peripheral nerve injury treatment. hDPSCs should be considered as a good choice for peripheral nerve injury therapy, because they have better potential to differentiate into neural and glial cells than stem cells coming from other sources through the expression of neuronal makers and wide range of neurotropic factors secretion. Unique properties of hDPSCs, such as high proliferation rate, trophic factors expression and stronger neuroprotective effects, indicate that these stem cells may be beneficial in neural disease therapy.

Keywords: dental pulp stem cells, neurodegenerative diseases, peripheral nerve injury, stem cell therapy

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Abbreviations: **AD** – Alzheimer's disease; **ALP** – alkaline phosphates; **BDNF** – brain-derived neurotrophic factor; **bFGF** – basic fibroblast growth factor; **BM-MSCs** – MSCs derived from bone marrow; **CNTF** – cytokine ciliary neurotrophic factor; **DFSCs** – dental follicle stem/progenitor cells; **DMP-1** – dentin matrix protein-1; **DSCs** – dental stem cells; **DSPP** – dentin sialophosphoprotein; **EGF** – epidermal growth factor; **FGF-β** – fibroblast growth factor–β; **GDNF** – glial cell line-derived neurotrophic factor; **GF-1** – insulin-like growth factor; **GFAP** – glial fibrillary acidic protein; **GFAP** – glial fibrillary acidic protein; **GMP** – Good Manufacture Practice; **GMSCs** – gingiva stem cells; **hDPSCs** – human dental pulp stem cells; **HGF** – hepatocyte growth factor; IL-6, IL-8, IL-10 – interleukin 6, 8, 10; **MAP-2** – micritubule-association protein 2; **MAP-2** – microtubule-associated protein-2; **MPTP-1**-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; **MSCs** – mesenchymal stem cells; **NF** – neuorofilament; **NGF** – nerve growth factor; **NT 3** – neutrophin 3; **OA** – okadaic acid; **OCN** – osteocalcin; **OPN** – osteopontin; Osx – osterix; **PD** – Parkinson's disease; **PDLSCs** – periodontal ligament stem cells; **PGE2** – prostaglandin E2; **SCAP** – stem cells from apical papilla; **SCI** – spinal cord injury; **SHED** – stem cells from human exfoliated deciduous teeth; **Shh** – sonic hedgehog; **TGF-β** – transforming growth factor-β; **TNF-α** – tumor necrosis factor-α; **VEGF** – vascular endothelial growth factor.

INTRODUCTION

Stem cell-based therapies have been intensively studied as a possible treatment of central nerve system disorders, i.e. Alzheimer's disease (AD), Parkinson's diseases (PD), stroke, spinal cord injury (SCI), and peripheral nerve injury [34, 58]. Mesenchymal stem cells (MSCs) coming from different sources were applied in the repair of nerve damage and regeneration of neurodegenerative diseases [62]. MSCs may be isolated from various human tissues including: skin, bone marrow, brain, adipose tissue and dental pulp of children and adults [61]. MSCs derived from bone marrow (BM-MSCs) are the most widely studied *in vitro* and *in vivo* experiments [60, 61]. However, pain and morbidity accompanying MSCs obtained from bone marrow forced clinicians and scientists to search for alternative donating organs or compartments as a source of MSCs [58]. In this sense, dental-related tissues are currently being proposed as one of the most promising non-invasive sources of human stem cells [34, 58]. Currently, there is growing evidence that dental stem cells (DSCs) have many similarities to BM-MSCs and evidently prevail over costly and invasive techniques required for other adult stem cells isolation [5, 34].

Dental pulp is a soft connective tissue of the tooth, united with the mineralized dentin and containing a heterogeneous population consisting of fibroblasts, endothelial cells, neurons, odonto-osteoprogenitors and inflammatory cells [5, 34, 39]. There are six types of stem/progenitor cells determined in dental-related tissues: dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), dental follicle stem/progenitor cells (DFSCs), periodontal ligament stem cells (PDLSCs) and gingiva stem cells (GMSCs); DPSCs, SHED and SCAP are referred to as dental pulp-related stem cells, whilst PDLSCs and DFSCs – as periodontal-related stem cells [39]. Although the above various mesenchymal stem cell populations exist in teeth, these groups are similar to one another and they also demonstrate specific characteristics relevant to each population [5, 39]. A comparison of biological features of dental stem cells coming up from different dentin tissues is presented in Table 1.

Due to the evidence proving that – in contrast to the other dental tissues – dental pulp is significantly richer in stem cells, hDPSCs have become "a promised land" for future clinical application [5, 7, 34, 62]. It has been found that hDPSCs have some features resembling MSCs characteristics, including fibroblast morphology with selective adherence to solid surfaces and formation colonies *in vitro* [5, 39, 62]. hDPSCs are easily accessible and possess higher proliferation capacity than ordinary MSCs [39, 62]. A considerable amount of data has shown that hDPSCs are characterized by their negative expression of hematopoietic antigens (e.g., CD45, CD34, CD14, CD19, HLA-DR) and positive of mesenchymal stem cells markers (e.g., CD105, CD90, CD44, CD29, CD73, STRO-1) [5, 7, 39, 40, 62]. Moreover, some of the pluripotent stem cell markers, such as Oct4, Nanog, Sox-2, SSEA and c-Myc have been expressed in hDPSCs [5, 62]. Apart from stemness markers, hDPSCs also express bone markers such as dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), osterix (Osx), osteocalcin (OCN), osteopontin (OPN) alkaline phosphates (ALP) and type I collagen [5, 7, 64]. Additionally, hDPSCs express neural markers such as β-III tubulin, microtubule-associated protein-2 (MAP-2), and glial fibrillary acidic protein (GFAP) [34, 62]. Moreover, dental pulp stem cells secrete many factors, such as immunomodulatory, anti-inflammatory, anti-apoptotic, anti-angiogenic regulatory and neurotrophic [5, 34, 62]. Biomarkers and factors expressed by hDPSCs are shown in Table 2. In fact, because hDPSCs populations are heterogeneous and consist of mixed subpopulations with different

Table 1. Biological features of dental stem cells coming up from different dentin tissues [34, 35, 58, 62]

	DPSCs	SHED	PDLSCs	DFPCs	SCAPs	GMSCs
Location	Permanent tooth pulp	Exfoliated decidous tooth pulp	Periodontal ligament	Dental follicle of developing tooth	Apical papilia of developing root	Gingival tissue
Immunoreactivity Positive biomarkers	STRO-1, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD117, CD146	STRO-1, CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166	STRO-1, CD10, CD13, CD26, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD166	STRO-1, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105	STRO-1, CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166	STRO-1, CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166,
Negative biomarkers	CD14, CD19, CD24, CD34, CD45, HLA-DR	CD14, CD18, CD19, CD24, CD34, CD45	CD14, CD34, CD40, CD45, CD80, CD86, HLA-DR	CD34, CD45, HLA-DR	CD18, CD34, CD45, CD150	CD34, CD45, HLA-DR
Multipotentialy	odontoblast, osteoblast, chondrocyte, myocyte, corneal epithelial cells, melanoma cells, iPS	odontoblast, osteoblast, chondrocyte, myocyte, neurocyte, adipocyte, iPS	odontoblast, osteoblast, chondrocyte, neurocyte, cementoblast	odontoblast, osteoblast, neurocyte,	odontoblast, osteoblast, neurocyte, adipocyte, iPS	osteoblast, chondrocyte, adipocyte, endothelial cells, neural crest stem-like cells
Proliferation rate	moderate	high	high	high	high	high
Heterogeneity	+	+	+	+	+	+
Tissue repair: regeneration	bone, neuro-myogenic, dentin-pulp	bone, neuro-myogenic, tubular dentin	bone, peridontal, root formation	bone, peridontal	bone, neuro-dentin--pulp, root formation	periodontal, peripheral nerve system

phenotypic and biological properties, and markers profiles, these stem cells have been frequently used in the regeneration of many tissues including neural tissue [3, 5, 34, 39, 62]. Experimental studies revealed that hDPSCs can be differentiated by modulation with growth factors, transcriptional factors, extracellular matrix proteins and receptors into mesodermal and non-mesodermal tissue cells, including osteoblasts, odontoblasts, adipocytes, chondrocytes, cardiomyocytes, neuron cells, corneal epithelial cells, hepatocytes and melanocytes [5, 34, 45, 62, 64]. According to International Society for Cellular Therapy (ISCT), three criteria, i.e. adherence to plastic, specific surface antigen, trilineage mesenchymal differentiation, should be used to define MSC subpopulation [13]. In our experimental studies, identification and characterization of hDPSCs was confirmed by stem cells markers expression, adherence to plastic dishes and differentiation potential toward osteoblasts and chondrocytes. The status of hDPSCs differentiated into osteoblasts and chondrocytes was confirmed by the expression of specific proteins, such as osteopontin, osteocalcin for osteoblast andand collagen, aggrecan for chndrocytes is presented in fig. 1F–H. As is widely mentioned in literature, considering hDPSCs use in therapy two their biological features are essential to obtain mature differentiated cells. First of all, high hDPSCs differential potential, secondly, differentiated cells biomarkers expression [25, 34, 40, 45, 58]. Recent data indicates that hDPSCs possess high neurogenic potential and express a high level of neuronal markers and neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) [25, 28, 29, 34]. Therefore, these dental stem cells seem to be good candidates used in neural diseases therapy and nerves regeneration [34, 58, 62].

EXPERIMENTAL STUDIES ON HDPSCS NEURONAL DIFFERENTIATION

hDPSCs, which arise from the cranial neural crest and express a high level of neurotrophic markers, seem to be an attractive subpopulation of stem cells, which in the appropriate environment differentiate towards functional neurons [25, 34, 58]. Several protocols have been developed to differentiate hDPSCs into dopaminergic neural cells [25, 45, 58]. These methods involve different growth factors and various supplements depending on phases of hDPSCs differentiation [45]. The growth factors include the following: epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), NGF, BDNF and GDNF [9, 18, 20, 26, 28, 29, 45, 65]. Additionally, neurogenic maturation of hDPSCs is achieved by the addition of sonic hedgehog (Shh), neurotrophin-3 (NT-3), heparin, retinoic acid, froskolin and culture supplement, such as B27 [18, 20, 23, 25, 26, 43].

The first study conducting hDPSCs differentiation into functional neurons using protocol based on fibroblast growth factors or epidermal growth factor signaling pathway was performed by Arthur et al. [6]. In another

Table 2. Markers and factors expressed by hDPSCs [5, 34, 35, 39]

Mesenchymal markers	Stemness markers	Neural markers	Neurotrophic factors	Immuno-modulatory factors	Anti-apoptotic factors	Angiogenic
CD13	OCT3/4	Nestin	NGF	PGE2	TNF-α	TGF-β
CD29	SSEA4	β-III tubulin	GDNF	IL-6, IL-8, IL-10		VEGF
CD44	NANOG	S100	BDNF	TGF-β		PDGF
CD146	SOX 2	NF	CNTF	HGF		IGF-1
CD166	STRO 1	GFAP	NT3			FGF-β
CD73		Synaptophysin				
CD90		MAP-2				
CD105						

NF – neuorofilament; **GFAP** – glial fibrillary acidic protein; **MAP-2** – micritubule-association protein 2; **NGF** – nerve growth factor; **GDNF** – glial cell line-derived neurotrophic factor; **BDNF** – brain--derived neurotrophic factor; **CNTF** – cytokine ciliary neurotrophic factor; **NT3** – neutrophin 3; **PGE2** – prostaglandin E2; **IL-6, -8, -10** – interleukina 6,8,10; **TGF-β** – transforming growth factor; **HGF** – hepatocyte growth factor; **TNF-α** – tumor necrosis factor α; **VEGF** – vascular endothelial growth factor; **PDGF** – platelet-derived growth factor; **FGF-β** – fibroblast growth factor β; **IGF-1** – insulin-like growth factor

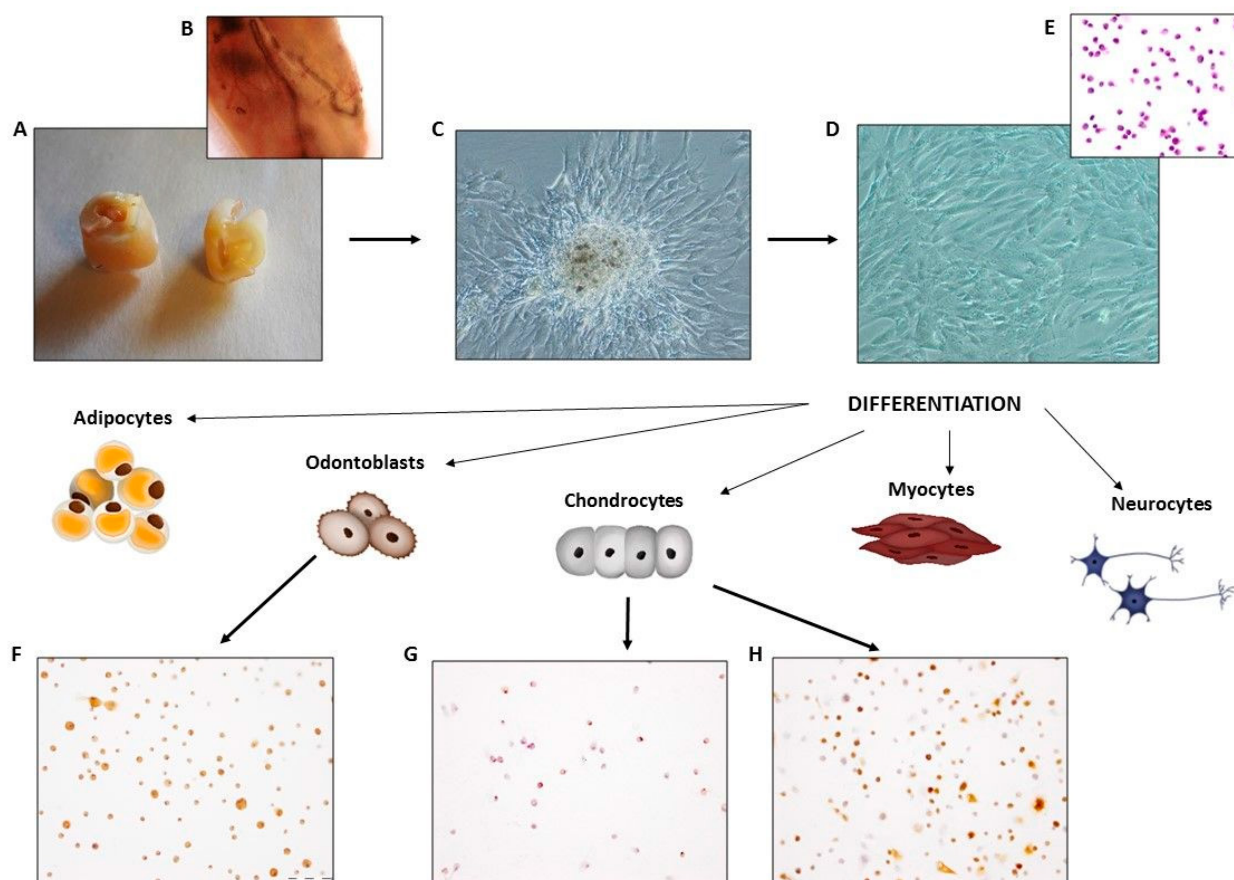

Fig. 1. Multilineage differentiation potential of hDPSCs isolated from adult tooth with fully developed roots. Extracted 3rd lower molar where the crown was separated from the root in the using the forceps soflex discs (A). The separated dental pulp tissue from extracted tooth showing vascularization (B). hDPSCs in the primary culture 7 days after seeding (C). 12 days after seeding (D). Morphological features of hDPSCs culture at 12th day of culture (E). Osteopontin expression in differentiated hDPSCs into osteoblast (G). Collagen type II (F) and aggrecan (H) expression in differentiated hDPSCs into chondrocytes. Fig. 1C, D both images were taken using invert microscopy magnification x 200, Figure 1E (hematoxylin–eosin staining, magnification x200), Fig. 1G–H (immunohistochemical staining, EnVision technique). The scale bar = 100 µm

study, the authors observed that during simultaneous activation of protein kinas C and cyclic AMP-pathways induced neural differentiation of hDPSCs [27]. The authors confirmed that hDPSCs differentiated into functional neurons by expression of neuronal markers, MAP-2 and GFAP [27].

There are reports showing that hDPSCs culture in Neurobasal Medium supplemented by EGF, bFGF or B27 supplement might form bipolar and stellate neuron-like morphology, which contains functional neurons confirmed by patch-clamp analysis of the voltage-gate Na+ an K+ channels [18, 27, 45]. In our preliminary experimental study, we observed that hDPSCs cultured in PSC Neural Induction Medium contains the following: Neurobasal Medium and Neural Induction Supplement (Gibco/Life Technology Cat No A1647801) differentiated toward neuron-like cells after 14 days of cultivation. For this study, we obtained the approval of the Ethics Committee of Wroclaw Medical University (decision number KB513/2019). Before differentiation, hDPSCs were positive for CD73, CD105, CD90, CD44, Stro-1 and HLA ABC antigens expression and negative for CD45, CD31, and HLADR expression. After differentiation, hDPSCs status was analyzed by β-III tubulin, nestin, GFAP, NeuN expression and evaluation of morphological features differentiated hDPSCs. The majority of differentiated hDPSCs showed high immunopositivity for nestin and β-III tubulin, whereas expression of stem cells markers significantly decreased. Morphological features of differentiated hDPSCs resemble neuron-like cells (Fig. 2A–E).

It was also reported that hDPSCs treated with BDNF, NT-4 and GDNF factors, might differentiate into spiral ganglion neuron-like cells showing functional neural activities [9, 22, 23, 35]. In another study, Gnanasegaran et al. [21] observed that hDPSCs differentiate into dopaminergic-like cells by multistage inductive protocols, whereas Singh et al. [52] showed that hDPSCs are induced by a two-step method to generate dopaminergic neurons: FGF2 first with addition of BDNF on the ninth day. Likewise, Chun et al. [11] and Gervois et al. [18] reported that hDPSCs could be differentiated into dopaminergic neural cells by forming a neurosphere. A recent study demonstrated that protocols used for hDPSCs differentiation make it possible to obtain a neuronal population of cells which shows increased glutamatergic and GABA-ergic markers and decreased dopaminergic and glial markers [10]. Routinely, the stage of hDPSCs neuronal differentiation is confirmed by the evaluation of such neural markers as GFAP, MAP--2, neural nuclei, synapsin I and neuron specific β-III tubulin [18, 25, 26, 43, 45]. Moreover, the standardization of hDPSCs differentiation protocols according to Good Manufacture Practice (GMP) is necessary. Based on solid performed experiments showing high neurogenic potential of hDPSCs, there seems to be strong evidence for using these stem cells for the treatment of central nervous system diseases and peripheral nerve injury in the future.

DPSCS IN CENTRAL NERVOUS SYSTEM DISEASE THERAPY

An experimental study in animal models has provided evidence for the advantages of dental stem cells-based therapy in central and peripheral nervous system diseases therapy [5]. A large amount of research has revealed the usefulness of hDPSCs in the treatment of main central nervous system diseases, including SCI, stroke, Parkinson's and Alzheimer's diseases [34, 58].

Spinal Cord Injury

A spinal cord injury leads to partial or complete loss of the body's sensory, motor and automatic function [33]. It may lead to the loss of movement, altered sensation, loss of bowel or bladder control, pain and difficulty in breathing caused by mechanical damage of nerve cells and blood vessels disruption or neuroinflammatory responses [15]. A new option for SCI therapy is the use of hDPSCs on account of their neural crest lineage and differential ability toward neuron-like cells [2, 34, 48, 58, 63]. Several studies have reported that hDPSCs transplantation recovered functionality of SCI [58, 63]. The grafted hDPSCs migrated and disseminated in the host spinal cord and started to differentiate into functionally neural cells [58, 63]. Neural repair mechanisms of hDPSCs in spinal cord injury have been analyzed by many investigators [2, 34, 48, 58, 63]. Yang et al. [63] found in rat model that hDPSCs after transplantation may differentiate into mature neural cells as neuron-like and oligodendrocyte-like cells that may promote axonal regeneration and tissue repair of spinal cord injuries. It was also shown that hDPSCs may reduce the inflammatory response by inhibition of interleukin-β expression and promotion of neurite regeneration by inhibition of Ras homolog gene family member A (RhoA) and decline in the rate of haemorrhage necrosis by reduced sulfonylurea receptor1 (SUR-1) [63]. Additionally, hDPSCs may protect Purkinje cells in cerebellar layers against 3-AP--induced neurotoxicity and inflammatory response [2, 33]. Experimental researches revealed that neuroregenerative mechanisms caused by hDPSCs include inhibition of the neural cells apoptosis in the injured spinal cord and promotion axons regeneration by stopping the expression of multiple axon growth inhibitors [58, 62, 63].

Stroke

Stroke can be manifested as cerebral ischemic due to long terms of insufficient blood supply leading to brain damage or even death [34, 62]. The therapeutic strategies that can be use are not effective enough [62]. The experimental therapy using hDPSCs transplantation into the ischemic areas in Sprague-Dawley (SD) rats promoted locomotor functional recovery and decreased infarct areas caused by differentiated hDPSCs into dopaminergic neurons and secretion of neutrophic factors [34, 42, 68]. It has been observed that hDPSCs play a protective role for astrocytes by reducing reactive gliosis and preventing free radical and IL-1β

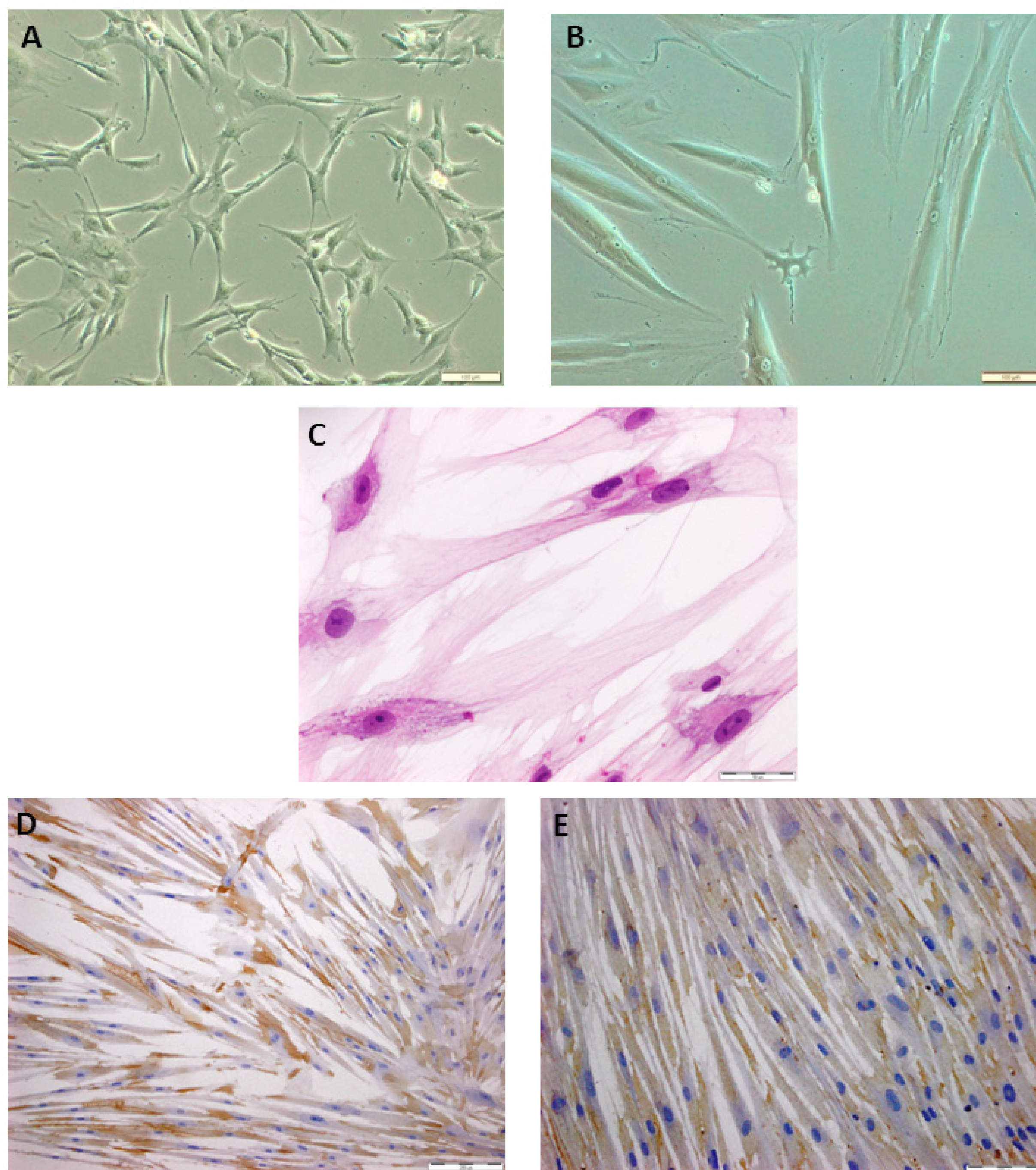

Fig. 2. hDPSCs isolated from dental pulp tissue taken from extracted 3rd molar tooth of 15-year-old patient and cultured in neural inductive medium towards early phase of neuron-like cells. hDPSCs before differentiation (A). Differentiated hPDSCs showing neuron-like morphology (B). Morphological features of differentiated hDPSCs represent features similar to neuron-like cells, many cells generate connection between themselves (C). Majority of cells showed nestin expression (D). Heterogeneous pattern of β-III tubulin expression visible in differentiated cells (E). Fig. 2A, B hDPSCs visualized by inverted microscope. The scale bar = 100µm. Fig. 2C (hematoxylin-eosin staining) the scale bar = 50µm, Fig. 2D, E (EnVision technique). The scale bar = 200µm and 100µm respectively Fig. 2D, E

secretion in *in vitro* ischemic model [34]. Recently, Nito et al. [42] maintained that human DPSCs reduced ischemic damage and improved functional recovery in a rodent ischemia model, which may relate to the modulation of neuro-inflammation during the acute phase of stroke. Therapeutic potential of intravenous administration of hDPSCs in a rat stroke model indicated that these stem cells may migrate and survive within a central nervous system lesion site, inducing differentiation of hDPSCs into neuron-like cells and replacement of lost neurons or paracrine-mediated supports of endogenous neuronal survival [37, 53, 67].

Parkinson's disease

Parkinson's disease (PD) is a progressive brain disorder caused by the loss of nigrostriatal dopaminergic neurons and is manifested by muscle rigidity, bradykinesia, resting tremor and postural instability [34, 62]. Chun et al. [11] found that human DPSC differentiate under neurogenic induction medium present neuron-like cells features and possess the ability to synthesize dopamine. hDPSCs may restore nigrostriatal dopaminergic neurons functions by reducing the secretion of proinflammatory factors (IL--1α, IL-1β, IL-6, IL-8, TNF-α) and by up regulating anti-inflammatory factors (IL-2, IL-4, TNF-β) [22]. Intrathecal delivery of hDPSCs into the 1-methyl-4-phenyl-1,2,3,6--tetrahydropyridine- (MPTP-) induced PD model promoted the recovery of behavioral function of cells [51].

Alzheimer's disease

Alzheimer's disease (AD) is an age-related chronic neuro-degenerative condition characterized by the loss of neurons, intracellular neurofibrillary tangles, and the deposition of insoluble β-amyloid peptides in the brain [34, 58]. The pathological changes in AD associated with dementia are the following: memory loss, cognitive impairment and linguistic disorders [34, 58]. Many types of stem cells have been used in AD therapy with different effectiveness [36, 58]. Experimental studies found that MSCs after transplantation in AD animal model showed neural regenerative effects, decreasing the number of amyloid plaques in the brain and inhibiting the secretion of inflammatory cytokines [36]. Many researchers suggest that hDPSCs have significant prevalence in AD therapy than MSCs from other sources [58, 59]. The therapeutic possibility of hDPSCs was studied using *in vitro* AD model [58, 59]. It was found that hDPSCs express many neutrophic factors, amyloid beta-degrading enzymes (NEPs) and anti-apoptotic factors, which have an impact on Alzheimer's disease therapy [1, 58]. Additionally, the experiment using an okadaic acid (OA) – induced *in vitro* AD model showed that hDPSCs could promote neural repair and regeneration by restoring the cytoskeletal structure and protect microtubule stability [58, 59]. Secretion by hDPSCs of various growth factors contributed to the inhibition of the phosphorylation of Tau protein and promoted neural stem cells proliferation [58]. hDPSCs can be induced to differentiate into dopamine expressing neuron-like cells and even as exosomes possess the ability to penetrate the blood-brain barrier and replace neuronal loss [58].

hDPSCs in peripheral nerve system disease therapy

Peripheral nerve injury is mainly caused by traumatic accidents or iatrogenic damage, which may result in physical disability [34]. Routine clinical treatment, including surgical solution, tends to choose the end-to-end/end-to-side neurorrhaphy to join the parts of damaged nerves [56]. In some cases, the patient has been offered nerve graft or active biomaterials, which might induce the regeneration of injured peripheral nerves [34, 56]. Up to now, autologous nerve grafting is the most recommended therapy for peripheral nerve deficits [56, 58]. However, this therapy has many disadvantages, such as donor nerve availability immunological response and morphometric mismatching [14]. Advancements in stem cell biological research and tissue engineering have given rise to the development of new strategies in the therapy and regeneration of peripheral nerve injury [16, 30, 47]. It was observed that hDPSCs share a common origin with peripheral nerve glial progenitor cells, and this feature makes these cells very valuable for peripheral nerve repair [47, 55]. Lately, encouraging results from different studies indicate that hDPSCs secretomes have a reparative and protective influence on axonal growth [8, 55]. Evidence from sciatic nerve injury model revealed that transplantation of Schwann-like cells induced from hDPSCs facilitated the regeneration of 15 mm sciatic nerve defect [47]. Another study preformed on sciatic nerve injury models showed that hDPSCs positive for STRO-1/Kit+/CD34+ are able to promote peripheral nerve repair by differentiating into Schwann cells precursors and secreting neurotrophic factors [8]. A recent study showed that single application of hDPSCs immediately after facial nerve crush injury in rat can promote a positive local effect on neuro-protection and remyelination in 2 weeks of treatment [46]. There are many reports in the literature on hDPSCs use in peripheral nerve therapy, but the results on hDPSCs effectiveness in peripheral nerve injury is controversial [17, 24, 35, 57]. Current results about the effectiveness of hDPSCs use in peripheral nerve injury therapy in *in vitro* and in vivo studies are presented in Table 3. As presented in Table 3, some studies found that after hDPSCs transplantation these stem cells were able to promote axonal growth and recovery of neuron function.

DPSCS COMBINED WITH 3D SCAFFOLDS FOR NEURAL REGENERATION *IN VIVO* MODELS

Many different strategies have been tried to find efficacious treatments for the nerve injuries. Currently, there are many basic studies showing extensive evidence of positive regenerative effects of hDPSCs combining with scaffold in neural regeneration [34]. Stem cell-based therapies and tissue engineering hold some promise as a novel strategy for tissues and nerve regeneration [66].

Table 3. The effect of human DPSCs use in peripheral nerve repair or regeneration both in vivo and *in vitro* studies

Author (publication year)	Type of experiments	Source of stem cells	Target tissues	Study model	Outcome
Carnevale et al. 2018 [8]	*in vivo*	hDPSCs STRO-1+/cKit +/CD34+expressing P75NTR, nestin, SOX-10	Sciatic nerve defect	rat model	hDPSCs promoted regeneration and functional recovery of sciatic nerve defects after injury
	in vivo	hDPSCs STRO-1+/cKit +/CD34+expressing P75NTR, nestin, SOX-10	differentiate into neuron-like cells	*In vitro* culturing of hDPSCs and their differentiation to neuronal cells	Under appropriate conditions, the cells differentiated into neuron-like cells
Kolar et al. 2017 [28]	*in vivo*	hDPSCs SCAP, DPSCs, PDLSC	10 mm nerve gap defect in a rat sciatic nerve	rat sciatic nerve injury model	hPDSCs significantly enhanced axon regeneration of peripheral nerve after two weeks from transplantation
	in vivo	stimulated human SCAP, DPSCs, PDLSC	Differentiated human neoroblastoma SH-SY5Y cell line	*In vitro* neurite outgrowth assay	Quantification of the neurite outgrowth showed that unstimulated and stimulated human DPSCs and PDLSC increased both the percentage of cells producing neurites and the total neurite outgrowth length
Sanen et al. 2017 [47]	*in vivo*	SCs derived from differentiated hDPSCs	15 mm rat sciatic nerve defects	Rat sciatic nerve injury model	Immunohistochemical and ultrastructural analysis revealed in-growing neurites, myelinated nerve fibres and blood vessels along the construct
	in vivo	SCs derived from differentiated hDPSCs	Human microvascular endothelial cell line (HMEC-1)	Alamar Blue cell proliferation assay; Transwell migration assay; Tube formation assay	The endothelial cell line HMEC-1 had proliferated significantly more in the presence of conditioned medium from hDPSCs and differentiated hDPSCs compared with those in control medium
Hei et al. 2017 [24]	*in vivo*	Schwann-like cells derived from hDPSCs	3 mm-wide crush injury was inflicted at a distance of approximately 10 mm from the mental foramen	Male Sprague-Dawley rats crush-injury site	Schwann-like cells, hDPSCs improved peripheral nerve regeneration
Ullah et al. 2017 [57]	*in vivo*	differentiated neuronal cells from hDPSCs	5 mm gap sciatic nerve transection	rat model	Transplantation of the undifferentiated hDPSCs could exhibit sufficient peripheral nerve regeneration potential
Geng et al. 2017 [17]	*in vivo*	hDPSCs	Differentiation of hDPSCs	*in vitro* model	Resveratrol induced hDPSCs differentiation into neuroprogenitor cells

Tissue engineering involves three essential components: appropriate cells, suitable three-dimensional (3D) scaffolds and inductive morphogenic signals to regenerate tissues and restore normal organ function [34]. Scaffolds are the membranes whose task is to carry the cells, induce proper microenvironment, and support tissue regeneration. Natural polymers are commonly used in their production, such as collagen, chitosan, silk, alginate, hyaluronic acid; synthetic materials, such as polylactic acid polyglycolic acid, polyethylene glycol; ceramic materials, such as tri calcium phosphate, biphasic calcium phosphate, calcium silicate [5, 32]. These materials were chosen due to their biocompatibility, bioactivity, supporting cells growth and differentiation. Moreover, the scaffold should have mechanical properties according to those of the tissue to be regenerated. In addition, the scaffold's pore architecture should allow and promote cell migration, proliferation, as well as diffusion of nutrients, oxygen and wastes [4]. As previously mentioned, hDPSCs share many common features with neural stem cells. Therefore, the combination of dental stem cells with biocompatible material exhibits great promise in neural tissue regeneration.

SCI therapies are mainly based on the use of dental stem cells transplanted in rat models in combination with different scaffolds. We focused on reports that showed the positive impact of DPSCs combined with different

materials on spinal cord repair. Zhang et al. [66] transplanted hDPSCs combined with chitosan scaffold into SCI rat model. Their findings showed that secreted neurotrophic factors, such as BDNF, GDNF, NGF, and NT-3 from transplanted hDPSCs, promote motor functional recovery and inhibit cell apoptosis after SCI [66]. The thermosensitive heparin-poloxamer (HP) hydrogel containing bFGF is the next material used with hDPSCs to regenerate the rat spinal cord [33]. The authors revealed that their biomaterial had more impact on neuronal regeneration, functional recovery, and tissue repair than transplanted HP hydrogel with bFGF-alone or hDPSC-alone strategies [33].

In peripheral nerve regeneration in rat models, scaffolds were used to regenerate facial nerve and sciatic nerve injuries. Sasaki et al. [49, 50] used rat DPSCs in polylactic glycolic acid (PLGA) tubes complex and also silicone tube containing rat DPSCs in type I collagen gel to regenerate injured rat facial. After two months post-transplantation, they observed that the regenerated nerves contained myelinated fibers and blood vessels. The authors confirmed the effectiveness of their bioimplant used in facial nerve defects therapy by analyzing cell nerve functionality, using electrophysiological methods [49]. Regarding sciatic nerve lesions, Sanen et al. [47] differentiated hDPSCs toward Schwann cells and loaded into collagen conduits. The authors confirmed the regenerative potential of hDPSCs by regeneration of critical (15 mm) sciatic nerve gaps. Qiao and colleagues repaired 1 cm purebred rabbit sciatic nerve defects using acellular nerve covered with homologous DPSCs [44]. An interesting study was performed by Spyridopoulos et al. [54], who used collagen conduits with autologous DPSCs to repair nerves in pigs. Their experiments proved that nerves where DPSCs were injected exhibited morphological and functional recovery [54]. Based on these data, we can suppose that DPSCs combined with biomaterials have a prosperous future in regenerative medicine for nerve repair.

FUTURE PERSPECTIVES OF HDPSCS USE IN NEURONAL DISEASES THERAPY

Only a few clinical studies were performed on oro-maxillo-facial bone repair [12, 19, 38]. Human autologous DPSCs were successfully used in patients with periodontal bone defects, post-third molar extraction defects and pulp regeneration [12, 31, 41]. HDPSCS were used by Li et al. [31] in the repair of human periodontal intrabone defects and after 9 months X-ray images results revealed the presence of a high-density osteoid. D'Aquino et al. [12] produced an effective biocomplex consisting of hDPSCs and collagen sponge, which was used for the regeneration of oro-maxillo-facial bone repair. The authors demonstrated excellent bone neoformation at the injury site one year after hDPSCs grafting [12], whereas Giuliani et al. [19] seeded hDPSCs onto a collagen sponge and assessed the stability and quality of the regenerated bone and vessel network three years after grafting intervention [19]. The authors found that the regenerated tissue from the graft site was composed of a fully compact bone with a higher matrix density [20]. Similarly, Monti et al. [38] observed bone regeneration after hDPSC transplantation into patients with mandibular defect. The generation of well-differentiated bones with structure resembling Haversian canals was observed by the authors [38]. Recently, a human DPSCs autologous transplantation in pulpectomized teeth was performed by Nakashima et al. [41] in a pilot study on five patients. No adverse events or toxicity were observed, and complete pulp regeneration was achieved after 24 weeks in all examined patients [41].

Clinical studies have revealed the therapeutic potential of hDPSCs in dentistry, which ecnourages researchers to study hDPSCs application in neural regeneration and repair. Furthermore, the advantage of hDPSCs is the fact that they can be obtained safely and easily without significant negative symptoms for the donor, which makes them a good biological material in the therapy of the injured nervous system. Moreover, the high neuro-regenerative potential of hDPSCs suggests that these stem cells seem to be good candidates for the therapy of central nervous diseases and peripheral nerve injury. Different growth factors which may have either positive or negative impact on the neurogenic differentiation capacity of hDPSCs should be taken into account in the treatment. As shown in this review, previous studies have shown that hDPSCs may be a useful tool for neurological disease therapy. However, the therapeutic effects of hDPSCs require more *in vitro* and *in vivo* research. Likewise, further studies are needed to test the application of hDPSCs in clinical trials, but there are still numerous obstacles that need to be overcome.

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