Origin and Clinical Applications of Neural Crest-Derived Dental Stem Cells

Yuan ZHU, Ping ZHANG, Ran Li GU, Yun Song LIU, Yong Sheng ZHOU

Over the past few decades, scientific research into neural crest-derived stem cells has progressed rapidly. The migration and differentiation of neural crest-derived stem cells has been an interesting area of research. Stem cells within teeth originating from the embryonic neural crest have attracted increasing attention in clinical and scientific research because they are easy to obtain and have superb stemness. The stem cells within the teeth include dental pulp stem cells (DPSCs), dental follicle stem cells (DFSCs), stem cells from apical papilla (SCAPs), stem cells from human exfoliated deciduous teeth (SHEDs), and periodontal ligament stem cells (PDLSCs). To date, there have been several interesting studies focusing on dental pulp regeneration, neural regeneration and the revascularization for therapeutic applications.
Key words: *dental stem cells, dentine/pulp engineering, neural crest cells, neural regeneration, revascularization*

Because it is difficult for the dental pulp and nerves to regenerate, there is currently no effect treatment for pulpitis and nerve injury. Recently, tissue engineering, including cells, growth factors and scaffolds, has become a popular therapeutic approach for cartilage and bone tissue construction, blood vessel, nerve tissue, skin, and oral tissue engineering. In addition, a vascular network can also provide oxygen and nutrients for transplanted cells. Cell-based therapy is a fundamental part of regenerative medicine. The cell types used for clinical trials are mainly hematopoietic cells and mesenchymal stem cells, and the treatment targets of cell-based therapies are oncology, cardiovascular, neurological, and autoimmune diseases; however, the majority of cell-based therapy clinical trials are in Phase I.

A major focus of regenerative medicine and tissue engineering involves the use of stem cells. Recently, increasing research into dental stem cells has made it a hotspot in stem cell research. In 2000, scientists first showed that there are stem cells in the dental pulp[1]; subsequently Miura et al identified SHEDs in 2003[2]. Subsequently, PDLSCs were identified from human periodontal ligament in 2004[3], and then DFSCs were isolated from human dental follicles[4]. Soon afterwards, Sonoyama et al first identified SCAPs from the apical area of extracted teeth in 2006[5].

These dental stem cells could undergo dentinogenic, osteogenic, chondrogenic, and neurogenic differentiation *in vitro* and have been widely used in tissue regeneration. DPSCs, SHEDs and SCAPs are used for bone regeneration, tooth tissue regeneration, and nerve regeneration *in vivo*. PDLSCs and DFSCs have mainly been investigated for the formation of periodontal tissue structure. The use of these stem cells is a promising strategy for the clinical treatment of many diseases; however, there are some controversial and doubts about the precise origin and clinical applications of dental stem cells. Hall claimed that vertebrate embryos have four germ layers and vertebrates are not triploblastic, but quadroblastic[6]. This viewpoint is different from the germ-layer theory. The neural crest-derived dental stem cells are featured in tissue engineering and regeneration; however, these cells have only been used in

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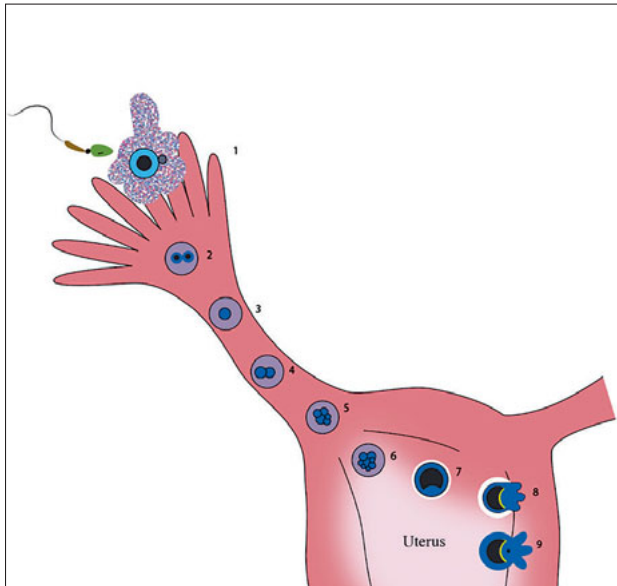
Fig 1 The migration of the zygote. The zygote undergoes cleavage in the first week of embryo development, and then implants in the uterine endometrium and becomes bilaminar. 1: The sperm and egg cell meet in the ampullar portion; 2 – 3: Sperm and egg cell membranes fuse and form a zygote, life begins at the time when the sperm and egg cell fuse; 4 – 6: The zygote undergoes cleavage as it travels down the oviduct and enters the uterus. The morula (number 6 in the figure) is a solid mass of blastomeres that forms when the zygote splits, and then develops into the blastocyst; 7: The blastocyst is a very early-stage embryo that is not yet implanted into the uterine endometrium; 8 – 9: The blastocyst hatches from the zona pellucida and then implants in the uterine endometrium. The trophoblast at the embryonic pole of the blastocyst proliferates to form the invasive syncytiotrophoblast.
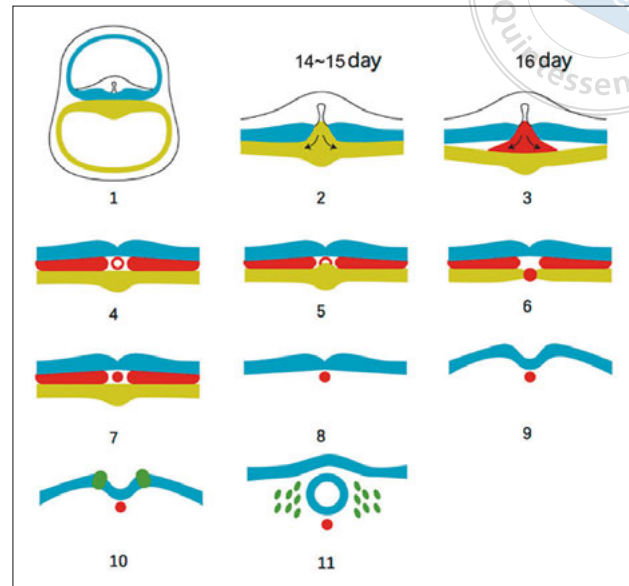

Fig 2 The formation of the trilaminar embryonic disc and neural crest cells. 1: Bilaminar embryonic disc. A primitive streak forms along caudal midline of the bilaminar embryonic disc, and its cranial end expands as a primitive node, which is the position of future oropharyngeal membrane; 2 – 3: Cells ingress and displace the hypoblast to form the definitive endoderm. Soon after, ingressing cells migrate and proliferate between the epiblast and the endoderm to form the mesoderm; gastrulation converts the bilaminar embryonic disc into a trilaminar embryonic disc; 4 – 7: The notochordal process is formed by the extended mesoderm on the 17th day and is hollow. Subsequently, the notochordal process fuses with the endoderm, with the fused position being called the notochordal plate. The notochordal process then separates from the endoderm and changes into a solid rod called the notochord on day 22; 8 – 11: The notochord process and notochord induce the ectoderm to thicken to form the neural plate (NP). The groove gradually deepens and ultimately the folds to meet in the middle line, which converts the groove into a closed tube. The interface between the surface ectoderm and the roof plate of the neural tube is the neural crest. The neural crest cells then migrate to different locations and differentiate into different cells and tissues.

cellular and preclinical studies, and have not yet been studied clinically.

In the current review, we discuss our current understanding of the origin and clinical applications of different neural crest-derived dental stem cells.

Origins of the neural crest and neural crest stem cells

The neural crest: The fourth germ layer

As we all know, the neural crest is a group of transient embryonic cells that were first described as the "Zwischenstrang", because of its origin between the epidermis

and the neural tube in the neurula-stage chick embryo. Then it was named "neural crest". Since then, different theories about the neural crest have been proposed, but the most accepted theory was the germ layer theory. In 1817, it was indicated that the blastoderm could develop into the three germ layers, an upper 'serous', a lower 'mucous', and a middle 'vessel' layer. Subsequently, the findings were formulated and expanded that vertebrate embryos were built on this three-layered plan. Later, the germ layer theory was extended from ontogeny into systematics and divided animals into homoplastica, diploblastica, and triploblastica, based on their number of germ layers.

A large number of cells derived from the ectoderm and mesoderm can be produced by the neural crest,

such as neurons, smooth muscle, bone, cartilage, and endocrine cells. Because of this, the three germ layers recognised for almost 180 years since Karl von Baer proposed the germ layer theory could be replaced by four germ layers. Hall claimed that vertebrate embryos comprised four germ layers and that vertebrates were not triploblastic, but quadroblastic[7]. The ectoderm and endoderm are the primary germ layers that appear earliest in the embryo. The mesoderm is a secondary germ layer because it arises following the ectoderm and endoderm's inductive interactions[8]. The mesoderm is recognised as a germ layer because it has the potential to differentiate into mesodermal cell and tissue types. The neural crest arises from the ectoderm, and can give rise to different types of cells and tissues, just like the mesoderm. Mesoderm can differentiate into different cell types. The neural crest can produce more types of cells and tissues than the mesoderm, thus the neural crest can qualify as a germ layer[8]. Thus, the neural crest should be regarded as the fourth germ layer and verte-brates are not triploblastic, but quadroblastic.

Neural crest stem cells (NCSCs)

The neural crest is an ephemeral structure that appears in the early development of embryos. As shown in Fig-ure 1, the zygote undergoes cleavage in the first week of embryo development. On day 5, the blastocyst implants in the uterine endometrium. The embryonic disc is bilaminar, consisting of epiblast and hypoblast layers at 7 days. By 14 days, the embryo expands, and the bilaminar embryonic disc is suspended in the chori-onic cavity by a thick connecting stalk. Along the cau-dal midline of the bilaminar embryonic disc, a primi-tive streak forms on day 15 and its cranial end expands as primitive node, which is the position of the future oropharyngeal membrane. Epiblast cells then ingress along the primitive streak, and displace the hypoblast to form the definitive endoderm. Soon after, ingressing cells migrate and proliferate between the epiblast and the endoderm, then form the mesoderm. Gastrulation then converts the bilaminar embryonic disc into a trilaminar embryonic disc and the embryo is referred to as a gas-trula. By day 17, a notochordal process is formed by the mesoderm, which extends cranially from the primitive node. At the same time, the mesoderm becomes the par-axial, intermediate, and lateral plate mesoderm from the midline to the lateral. The lateral plate mesoderm then begins to split into two layers, the splanchnic mesoderm and the somatic mesoderm; some scattered mesoderm cells are referred to as the mesenchyme. The paraxial mesoderm forms the head mesoderm and somites in the
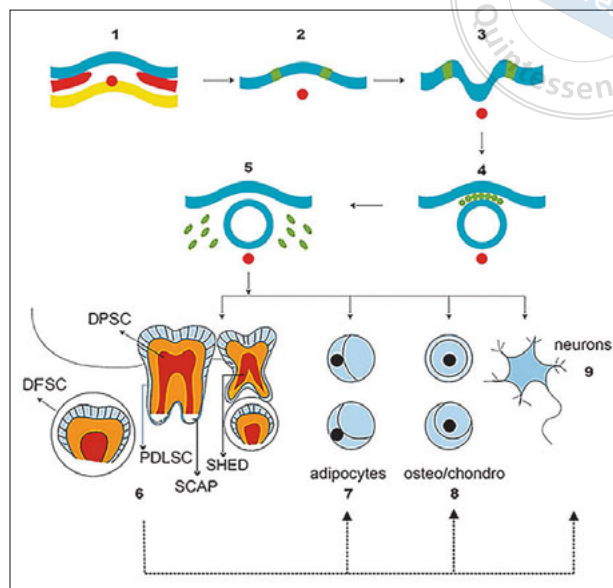

Fig 3 Neural crest cells give rise to multiple cell types and demonstrate the origin and differentiation potentials of dental stem cells. 1 – 5: The formation of neural crest cells; 6: Dental stem cells; the dotted arrow shows the differentiation potential of dental stem cells; 7: Adipocytes; 8: Osteoblasts and chon-drocytes; 9: Neurons.

future head and trunk regions, the intermediate meso-derm forms the major organs of the urogenital system and the lateral plate mesoderm forms the body wall and the wall of the gastrointestinal system[9] (Fig 1).

The notochord is formed by the extended mesoderm. Formation of the neural tube occurs during the neurula-tion, in which there are two ways to form a neural tube, primary and secondary neurulation. Generally speak-ing, neurulation is referring to primary neurulation. Neurulation can be subdivided into four stages: forma-tion, shaping and bending of the neural plate (NP), and closure of the neural groove (NG)[10]. The notochord induces the ectoderm to get thicker to form the NP at the end of the third week. The NP is surrounded on either side by the neural folds that are derived from the NP; the NG deepens and finally the folds meet in the middle line. The groove converts into a closed tube, forming the neural tube[11] (Fig 2).

The interface between the surface ectoderm and the roof plate of the neural tube is the neural crest. Neural crest cells undergo an epithelial-to-mesenchymal trans-formation (EMT), after which they migrate to differ-ent locations and differentiate into different cells and tissues. In vivo and in vitro experiments revealed that neural crest cells give rise to multiple cell types (Fig 3), including adipocytes[12], neurons[13], glias[14,15], smooth muscle, chondrocytes, osteoblasts[16], dentine (odonto-blasts)[8], and melanocytes[17]. Neural crest cells are
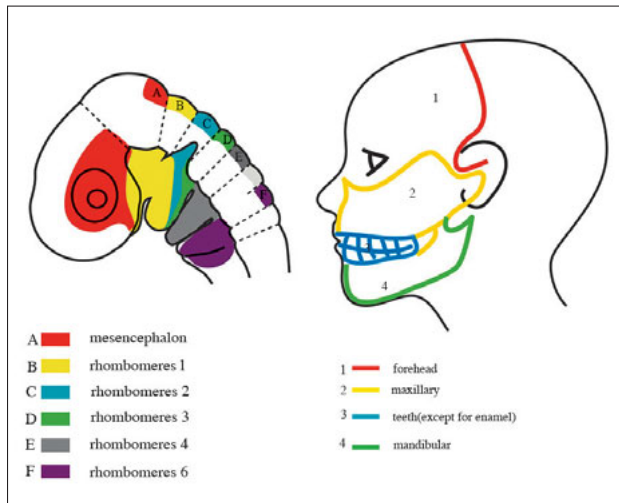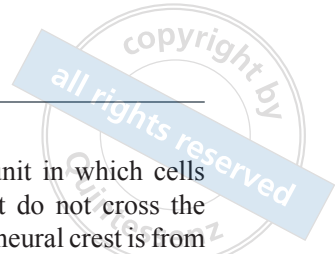
Fig 4 The migration of neural crest cells and the formation of face. The left picture shows the neural crest cells migrating from rhombomeres to pharyngeal arch, different colours show the different migratory routes. The right picture shows different colour regions refer to different neural crest cells.

defined because of their origin; they are multipotent and self-renewable[18-23]. Based on these points, neural crest cells are considered stem cells or stem cell-like[24,25].

The development of neural crest-derived dental stem cells

The neural crest can be divided into four major regions: Cranial, vagal and sacral, trunk, and cardiac neural crest[7]. The development of the craniofacial region is closely related to the cranial neural crest. The primordia of three primary brain vesicles are visible while the neural plate is thickened, including the prosencephalon, mesencephalon, and rhombencephalon (hindbrain). The rhombencephalon is subdivided into eight rhom-

bomeres, each is a developmental unit in which cells in a rhombomere can exchange, but do not cross the boundaries. The region of the cranial neural crest is from the caudal forebrain to the level of rhombomere six of the myelencephalon[9].

The neural crest cells originating from the midbrain and hindbrain regions form the cartilages in the pharyngeal arches[26]. Human embryos include six pairs of pharyngeal arches; the first four are well-defined, the first arch forms on day 22, the second and third on day 24, the fourth and sixth on day 29; however, the fifth arch does not form in human embryos or forms transiently and regresses sequentially[4].

The rhombencephalon neural crest cells migrate from rhombomeres (r) rl, r2, r4, r6, and r7, with little or no cranial neural crest cells apparent at the level of r3 or r5. Neural crest cells from the mesencephalon, r1, and r2 migrate into the first pharyngeal arch (mandibular arch), r4 into the second pharyngeal arch (hyoid arch), r6-r8 into pharyngeal arches 3-6, and pharyngeal arch 3 is mainly contributed by r6[27,28]. On day 28, the first pharyngeal arch (mandibular arch) is divided into two parts, the maxillary and mandibular prominences. The migrated neural crest cells in the frontonasal area arise from the forebrain and midbrain, and neural crest cells in the maxillary and mandibular prominences are from the midbrain and hindbrain (Fig 4).

The basic morphology of the face is established between the fourth and 10th weeks by the development and joining of five prominences: The frontonasal prominence, a pair of maxillary prominences, and a pair of mandibular prominences. In the sixth week, the nasal placodes form the nasal pits and the nasal processes. In the seventh week, media nasal processes fuse to form the intermaxillary process, and intermaxillary process give rise to the philtrum and the primary palate. The
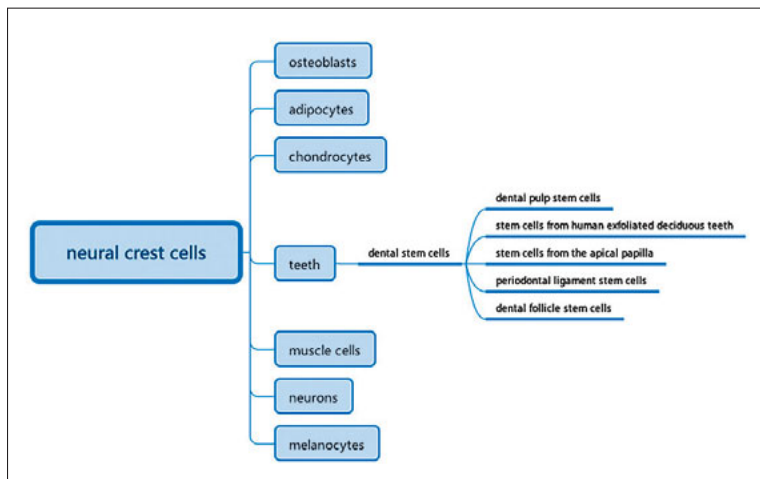

Fig 5 Types of stem cells derived from neural crest cells. The neural crest cells can differentiate into different kinds of cells and tissues, such as osteoblasts, adipocytes, chondrocytes, muscle cells, neurons, melanocytes and dental stem cells. Dental stem cells include DPSCs, DFSCs, SCAPs, SHEDs, and PDLSCs.

secondary palates are formed by the maxillary prominences in the eighth week; they form the majority of the palate and meet the primary palate at the incisive foramen. The maxillary and mandibular fuse with each other and in the eighth week, the prominence fusion is basically complete[29] (Fig 4).

The embryogenesis of teeth arises in the sixth week. Tooth morphogenesis involves a thickening stage, bud stage, cap stage, bell stage, and secretory stage[30]. There are a large number of stem cells within teeth that originate from the embryonic neural crest. These stem cells are easy to obtain and have superb stemness; therefore, they have attracted increased research attention. It has been demonstrated that cranial neural crest cells can differentiate into dentine matrix, pulp, cementum, dental papilla, odontoblasts, periodontal ligaments, and so on[8].

Different types of tooth derived stem cells

The neural crest cells can differentiate into different kind of cells and tissues, including dental stem cells (Fig 5). Dental stem cells, as described in Figure 6, include DPSCs, DFSCs, SCAPs, SHEDs, and PDLSCs. These dental stem cells are self-renewable and differentiate into other cells and tissues that originate from neural crest cells.

DPSCs

DPSCs were isolated from adult dental pulp in 2000[1]. These cells in the dental pulp form new odontoblasts and reparative dentine while undergoing some physiological lesions in teeth. *In vitro*, dental pulp stem cells can self-renew and differentiate into multiple cells, such as osteoblasts, odontoblasts, and neurons. *In vivo*, these cells can form a vascularised pulp-like tissue surrounded by odontoblast-like cells.

PDLSCs

PDLSCs were isolated and separated from the surface of the root and were identified by Byoung-Moo Seo et al in 2004[3]. The periodontal ligament (PDL) is a connective tissue between the cementum and alveolar bone socket, which supports teeth and contributes to nutrition, homoeostasis, and repair tissue. *In vitro*, PDLSCs express mesenchymal stem-cell markers in culture and can differentiate into cementoblast-like cells, osteoblasts, odontoblasts, and neuronal-like cells. *In vivo*, PDLSCs can generate cementum and PDL-like structures, suggesting that PDLSCs have a role in periodontal tissue regeneration.
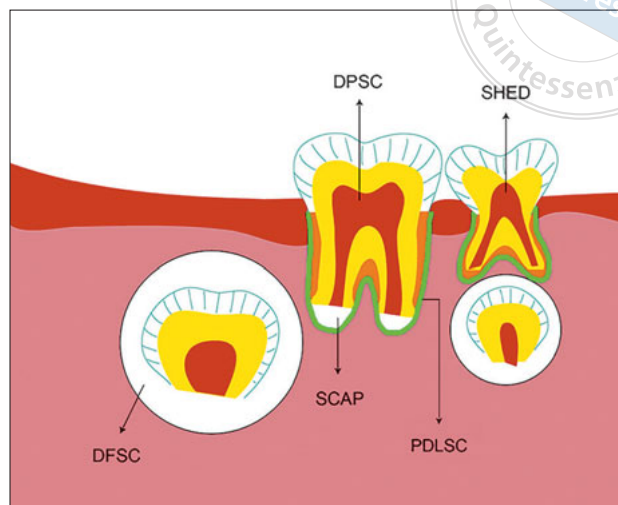

**Fig 6** Different types of tooth-derived stem cells.

DFSCs

DFSCs were isolated from human dental follicles and identified by Morsczeck et al in 2005[4]. The dental follicle is a connective tissue that surrounds the unerupted tooth. *In vitro*, DFSCs can differentiate into osteoblasts, adipocytes, and neuronal-like cells[31-33]. *In vivo*, DFSCs can build fibrous or rigid connective tissues after 8 weeks of differentiation lining Hydroxyapatite (HAP) residues.

SHEDs

Miura et al discovered these cells in 2003[2]. They used a technique very similar to DPSCs. SHEDs proliferate faster than DPSCs and can differentiate into osteoblasts and neuronal-like cells. SHEDs can form dentine-like structures *in vivo*, and could differentiate into neural tissue when injected into the dentate gyrus of the mouse hippocampus and expressed both neuronal and glial cell markers. These observations indicated that SHEDs could be used for dental pulp and neural regeneration.

SCAPs

Sonoyama et al identified SCAPS from the apical papilla of extracted third molars in 2006[5]. The apical papilla is involved in regulating root formation and later becomes the pulp. *In vitro*, SCAPs can differentiate into odontoblasts, chondrocytes and neuronal-like cells. *In vivo*, SCAPs can form dentine/pulp-like structures. SCAPs are cells from developing tissue; therefore, these cells may have a better potential for tissue regeneration than cells from mature tissues.
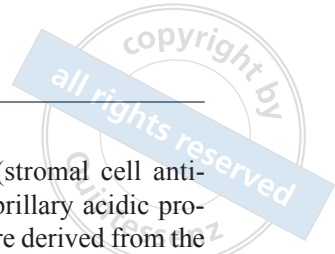
Potential therapeutic benefits of dental stem cells

Dentine/pulp engineering and regeneration

Dental pulp tissue is formed by odontoblasts, blood vessels, nerves, and connective tissue. Dental pulp regeneration is a process of biological tissue engineering to replace the damaged pulp tissue. The main challenges of dental pulp regeneration are revascularization and re-innervation, and the new dentine deposition by odontoblasts.

Although DPSCs, SHEDs, PDLSCs, SCAPs, and DFSCs can differentiate into odontoblasts within specific cultures *in vitro*, DPSCs, SHEDs, and SCAPs are frequently used for dentine-pulp regeneration because they are derived from pulp tissue or the precursor of pulp. DFSCs are isolated from dental follicles and contain precursor cells of the periodontal tissue, and PDLSCs are also a group of multi-potent stem cells that can differentiate into cementum/PDL-like tissue *in vivo*, so, DFSCs and PDLSCs are usually used in periodontal tissue regeneration to treat periodontal defects.

DPSCs can differentiate into multiple cell lineages, but they maintain their original properties as dental pulp cells because they are derived from dental pulp tissue. When Gronthos identified the DPSCs in 2000[1], they found that DPSCs could form a vascularised pulp-like tissue surrounded by odontoblast-like cells *in vivo*. In later research, DPSCs translated into animals could differentiate into odontoblasts and dentine/pulp-like tissues, sometimes with revascularization in some cultures. SHEDs are similar to DPSCs as they are both derived from dental pulp tissue. SHEDs can differentiate into multiple cells like DPSCs; however, SHEDs proliferate faster than DPSCs and fail to reconstitute a dentine/pulp-like complex, which might be explained by the fact that the developmental processes of deciduous teeth are different from permanent teeth[3]. SCAPs are isolated from the apical papilla, which is involved in regulating root formation and later becomes the pulp. SCAPs are capable of differentiating into the dentine/pulp-like complex, as the apical papilla is the precursor of pulp. SCAPs have a greater capacity for dentine regeneration than DPSCs[5]. Research into dentine/pulp regeneration is summarised in Table 1. Dentine/pulp regeneration research is still in the preclinical/animal experimental stage, and there have been few clinical trials.

Neural regeneration

DPSCs, SHEDs, PDLSCs, SCAPs, and DFSCs can differentiate into neuron-like cells and express important neuronal markers, including Stro-1(stromal cell antigen), nestin, c-FOS, GFAP (Glial fibrillary acidic protein), and βIII-tubulin because they are derived from the neural crest[44-51]. Research into neural regeneration is summarised in Table 2.

Those cells can differentiate into neuron-like cells; however, they do not mature into functional neuronal cells[50]. Activation of the cAMP (cyclic Adenosine monophosphate) and PKC (protein kinase C) signaling pathways can promote DPSCs to differentiate into functional neurons and glia cells[47]. Dental pulp regeneration comprises not only the regeneration of odontoblasts, but also the regeneration of the vital pulp of teeth. Dental pulp neural regeneration represents a protective response to temperature, chemical and mechanical stimulation to maintain the long-term survival of teeth. Although there has been little research into dental pulp neural regeneration, this approach will become a hot topic in pulp regeneration research in the future.

Central and peripheral nerve injuries are difficult to treat because the nervous system has a limited ability to repair damaged cells and tissues. But stem cells have regenerative effects on these defects. In this respect, dental stem cells have distinct advantages in that they can differentiate into neuron-like cells[45] and they are more easily obtained than iPS cells, bone marrow-derived mesenchymal stem cells and embryonic neuronal progenitors.

PDLSCs are associated mainly with periodontal tissue, rather than neural, regeneration; therefore, there has been little research about PDLSCs for therapeutic neural regeneration *in vivo*. DPSCs and SHEDs have been tested mainly for nerve regenerative therapies.

Revascularization

Functional tissue engineering and regeneration are challenging. Tissue regeneration requires a vascular network to provide oxygen and nutrients for transplanted cells[34,58].

Revascularization includes vasculogenesis and angiogenesis. Vasculogenesis refers to the mechanism of forming new blood vessels by the activation of vascular endothelial cell precursor cells; foetal vascular network formation occurs in this way[59]. Angiogenesis refers to the process whereby existing vessels proliferate to form new blood vessels. The important cytokines for vascular formation and regeneration are angiogenin (Ang), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), and platelet derived growth factor (PDGF). The applications of biomaterials cytokines and VEGF

Table 1 Dentine/pulp engineering and regeneration.

Cell	Cell source	Species	Host of Transplantation	Scaffold/Carrier	Result	Clinical Application	Ref.
DPSC	Third molars	Human	Immunocompromised beige mice	HA/TCP ceramic powder	Generate a dentine/pulp-like structure	Potential for dentinal repair	1
DPSC	First molar tooth buds	Rat	Adult rats	TGF–β1 soaked Millipore filter pieces	Tubular dentine formed; Differentiated into odontoblast	Inducing odontoblast differentiation and dentine formation	34
DPSC	Third molars	Human	SCID mouse	Human tooth slices (microtissue spheroids)	Pulp-like tissue and vessels regeneration	Regenerative endodontics	35
DPSC	Third molars	Human	Immunocompromised beige mice	HA/TCP ceramic powder	Generate dentine/pulp-like complex	Tissue regeneration	36
DPSC	Third molars	Human	Immunodeficient mice	Group4: Collagen scaffold impregnated with DPCSs and DMP1	Group 4 demonstrated several signs of tissue regeneration	Dentine regeneration	37
DPSC	Third molars	Human	Immunocompromised beige mice	HA/TCP ceramic powder	Regenerate a dentine–pulp-like structure	Forming ectopic dentine and associated pulp tissue *in vivo*	38
DPSC	Neonatal murine tooth pulp	Murine	Immunocompromised Rag1 null mice	HAp / TCP	Produce dentine-like structure	Tissue regeneration	39
SHED	Deciduous incisors	Human	Immunocompromised mice	HA/TCP	Induce bone formation, generate dentine, express neural markers	Stem-cell therapies: autologous stem-cell transplantation and tissue engineering.	2
SHED	Deciduous teeth	Human	Immunodeficient mice	Tooth slice/scaffolds	Differentiate into functional odontoblasts	Dental pulp tissue engineering	40
SHED	Deciduous teeth	Human	Immunodeficient mice	Tooth slice/scaffolds (PLLA, poly–L–lactide acid,)	Generate predentine in the pulp-like tissues	Dental pulp engineering	41
SCAP	Third molars	Human	Immunocompromised mice	HA/TCP	Dentine tissue formed on the surface of the HA/TCP along with connective tissue	Dentine regeneration, stem cell resource for tissue regeneration.	5
SCAP	Third molars	Human	Immunodeficient mice	A 3D and scaffold-free stem-cell sheet-derived pellet (CSDP)	Vascularised dental pulp-like tissue filled the root canal space	Dental pulp/dentine complex regeneration	42
SCAP	Third molars	Human	Immunodeficient mice	PLG(poly-D,L-lactide/glycolide) scaffolds	Pulp-like tissue regenerated	Pulp/dentine regeneration	43

HA/TCP, HAp/TCP: Hydroxyapatite/ Tricalcium Phosphate; MTA: mineral trioxide aggregate; TGF–β1: transforming growth factor–β1; SCID: severe combined immunodeficient; DMP1: dentine matrix protein 1.

Table 2 Neural regeneration.

Cell	Cell Source	Species	Host of Transplantation	Scaffold/Carrier	Result	Clinical Application	Ref.
DPSC	Third molars	Human	Chicken eggs	--	Express neuronal markers, acquire a neuronal morphology	Treat neurological disease(cell-therapeutic)	46
DPSC	Third molars	Human	Rat	--	Express neuron-specific markers, exhibit voltage dependent sodium and potassium channels	Cell therapy of degenerative disorders of the central nervous system	48
DPSC	Third molars	Human	SCI Rat	Chitosan scaffolds	Ameliorated locomotor recovery	Regenerative therapy	52
SHED	Deciduous incisors	Human	Mice	HA/TCP	Express neural cell markers	Treat neural tissue injury or degenerative diseases	2
SHED	Deciduous teeth	Human	rat	--	Neurodegeneration, nigrostriatal tract restoration.	Therapy for Parkinson's disease (PD)	53
SHED	Deciduous teeth	Human	ICR mice	--	Cognitive function improved	SHED-CM may provide a novel cell-free neuro-reparative therapy for Alzheimer's disease (AD).	54
SCAP	Third molars	Human	Rat	Fibrin hydrogels	Improve gait in spinally injured rats, reduce glial reactivity	Therapy for spinal cord injury	55
SCAP	A SCAP cell line called RP-89	Human	SCID mice	Matrigel	SCAP may be responsible for the chemical signal driving axons to target regenerated tissue	Neural regeneration	56
DFSCs	Third molars	Human	Sprague-Dawley rats	Electrospun PCL/PLGA material	Express oligodendrogenic lineage maker Olig2	Neural regeneration.	57

SCI: spinal cord injury; ICR: imprinting control region; SCID: severe combined immunodeficient; PCL: polycaprolactone; PLGA: poly D,L-lactic-glycolic acid.

Table 3 Revascularization.

Cell	Cell Source	Species	Host of Transplantation	Scaffold/Carrier	Result	Clinical application	Ref.
DPSC	Third molars	Human	SCID mice	Root slices 3D Microtissues	Enhanced vasculature	Securing blood supply	35
DPSC	Teeth from adults	Human	Immunosuppressed rats	Trimethylene carbonate and lactide polyglycolic acid	Vessels were found	Transplantation and surgical/clinical application in tissue repair.	60
DPSC	Porcine teeth	Porcine	SCID mice	Incisor roots	Produced higher blood flow and capillary density	Potential in ischemic disease therapy and pulp regeneration	61
DPSC	Third molars	Human	Chicken chorioallantoic membrane	Matrigel	Induce the formation of functional blood vessels	Tissue engineering	62
DPSC	Molar teeth	Mice	Mice	Modified Matrigel (MG) plug	Formed more mature tube-like structures, enhanced angiogenesis	Tissue regeneration.	63
DPSC SCAP DFSC	Third molars	Human	Chicken eggs	Matrigel	Predominant pro-angiogenic impact on endothelial migration and tube formation	Pulp regeneration and whole tooth engineering	64
SHED	Deciduous teeth; multicolony-derived murine pulp stem cells	Human; Murine	SCID mice; Swiss mice	Human tooth slice	FGF-2 priming enhances the angiogenic potential of SHED	Improving angiogenesis in tissue constructs	65
DPSCs	Third molars	Human	SCID mice	Peptide hydrogel PuraMatrix root fragments	Angiogenesis	Regeneration of vascular tissues; Dental pulp regeneration.	66
Pulp-CD31-; CD146-SP Cell	Porcine tooth germ	Porcine	SCID mice	--	Vasculogenic	Potential clinical utility to ameliorate ischemic disease; Pulp regeneration	67
DPSC	Third molars	Human	Nude rats	--	Increase the number of vessels and reduce infarct size	Treatment of ischemic diseases.	68
SHED	Deciduous teeth	Human	Immunodeficient mice	Tooth slice/scaffolds	Differentiate into functional odontoblasts	Dental pulp tissue engineering.	40
SHED	Deciduous teeth	Human	SCID mice	Tooth slice/scaffolds	Reduction in human CD31-positive blood vessels	Treatment for necrotic immature permanent teeth	69

genes for the induction of vascular growth, and angiogenic precursor cells for revascularization during tissue engineering, have good potential.

For pulp tissue regeneration, the formation of dentine needs vital pulp, which requires the vasculature to provide nutrients. Vascularised odontoblasts that can produce dentine and are innervated should be involved in regenerated pulp tissue. However, it is difficult for teeth to become vascularized because the apical canal for blood vessel entrance is small. There has been some research into revascularization (Table 3). As a result, the smaller the apical opening, the more difficult it is to regenerate blood vessels, and immature teeth are more suitable for pulp tissue regeneration.

Perspective

In this review, we discuss the neural and vascular regeneration of dental pulp tissue. There are some prospects for cell-based therapy for regenerative medicine:

- Dental stem cells are easily obtained and retain their proliferation ability; therefore, they will become a popular stem cell resource for regenerative medicine;
- Vital and functional dental pulp regeneration should include revascularization and re-innervation of the pulp, and the deposition of new dentine by odontoblasts;
- Dental stem cells can be used to repair damaged cells and tissues after central and peripheral nerve injuries, which are difficult to treat because the nervous system has limited ability to repair itself;
- The revascularization ability of dental stem cells can be used in functional tissue engineering and regeneration because tissue regeneration requires a vascular network to provide oxygen and nutrients for transplanted cells.

To date there have been only cellular and preclinical studies of the dental stem cells – no clinical studies have been published. In conclusion, it is important to take into consideration that dental stem cells have potential for effective therapy.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Drs Yuan ZHU, Ping ZHANG and Ran Li GU participated in literature collection and analysis; Drs Yuan ZHU and Ping ZHANG prepared the manuscript; Dr Yun Song LIU designed the study and revised the manuscript; Prof Yong Sheng ZHOU revised the manuscript.

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