Stem Cell Rev and Rep (2011) 7:161–171
DOI 10.1007/s12015-010-9155-0

A Journey from Dental Pulp Stem Cells to a Bio-tooth

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Published online: 27 May 2010

Abstract The ultimate goal of tooth regeneration is to replace the lost teeth. Stem cell-based tooth engineering is deemed as a promising approach to the making of a biological tooth (bio-tooth). Dental pulp stem cells (DPSCs) represent a kind of adult cell colony which has the potent capacity of self-renewing and multilineage differentiation. The exact origin of DPSCs has not been fully determined and these stem cells seem to be the source of odontoblasts that contribute to the formation of dentin-pulp complex. Recently, achievements obtained from stem cell biology and tooth regeneration have enabled us to contemplate the potential applications of DPSCs. Some studies have proved that DPSCs are capable of producing dental tissues in vivo including dentin, pulp, and crown-like structures. Whereas other investigations have shown that these stem cells can bring about the formation of bone-like tissues. Theoretically, a bio-tooth made from autogenous DPSCs should be the best choice for clinical tooth reconstruction. This review will focus on the location, origin, and current isolation approaches of these stem cells. Their odontoblastic differentiation and potential utilizations in the reconstruction of dentin-pulp complex and bio-tooth will be extensively discussed.

Keywords Dentin · Dental pulp · Stem cell · Odontoblast · Tooth regeneration

Abbreviations

ALP	Alkaline phosphatase
BMP2	Bone morphogenetic protein 2
BMMSCs	Bone marrow mesenchymal stem cells
BSP	Bone sialoprotein
CBFA1	Core binding factor alpha1
CD	Cell differentiation antigen
CNC	Cranial neural crest
CNCSC	Cranial neural crest stem cells
COLI	Type I collagen
DIF	Differentiation-inducing factors
DPSCs	Dental pulp stem cells
DSPP	Dentin sialophosphoprotein
FACS	Fluorescence activated cell sorting
FGF	Fibroblast growth factor
GDF11	Growth differentiation factor 11
HA-TCP	Hydroxyapatite-tricalcium phosphate
HSP	Hydrostatic pressure
iPS	Induced pluripotent stem cells
MACS	Magnetic activated cell sorting
OCN	Osteocalcin
PDLSCs	Periodontal ligament stem cells
SCAPS	Stem cells from apical papilla
SHEDs	Stem cells from exfoliated deciduous tooth
SP	Side population
STRO-1	Stromal precursor cell marker
TGF	Transforming growth factor

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Introduction

Since the discovery of adult dental pulp stem cells (DPSCs) in wisdom teeth in 2000 [1], several other types of DPSCs have been successively isolated from mature and immature dental pulps, including stem cells from exfoliated deciduous teeth (SHEDs) [2], stem cells from apical papilla (SCAPs) [3], and mesenchymal stem cells from tooth germs [4, 5]. Researches on their characteristics and differentiation potential have become one of the hot topics in the field of Dentistry. It is considered that these stem cells are undifferentiated mesenchymal cells present in dental pulp tissues and characterized by their unlimited self-renewal, colony forming capacity, and multipotent differentiation. The biological features of different DPSCs are summarized in Table 1.

Generally, DPSCs keep the common features of adult stem cells. (a) They are usually in a quiescent state in dental pulp, and can perform continuous cell division during tissue injury/regeneration [6–10]. (b) They have two types of growth patterns, i.e. the symmetric and asymmetric cell division. They can divide into two identical daughter stem cells by the symmetric cell division, or give birth to one unaltered daughter stem cell and one progenitor cell with limited self-renewal capacity by the asymmetric cell division [11]. (c) Clonal growth is another important feature of DPSCs [1, 12]. A single stem cell can generate a group of genetically identical offspring cells in vitro. (d) During the long-term passage culture, in vitro DPSCs may lose their stem cell nature including the self-renewal and multiple differentiation capacity [13–15]. This is mainly because the routine culture medium cannot completely simulate the in situ niches of these stem cells [16]. Additionally, due to the potent multi-differentiation ability, some growth factors in the medium or serum may induce their spontaneous differentiation in vitro [14, 17].

Huang et al. have shown that DPSCs can promote the proliferation and differentiation of neural cell in mice hippocampus, indicating that these dental stem cells may have a unique therapeutic potential in the central nervous system degeneration [18]. Ikeda et al. have proved that DPSCs can effectively prevent the further exacerbation of drug-induced rat liver fibrosis, and boost the recovery of liver function [19]. Intracardiac injection of DPSCs can reduce the area of myocardial infarction, improve ventricular function, and induce the revascularization around the injection region [20]. The transplantation of a tissue-engineered immature DPSCs sheet can reconstruct the corneal epithelium in an animal model [21]. When recombined with platelet rich plasma, DPSCs and SHEDs have the ability to form bone that is useful for the osseointegration of hydroxyapatite-coated dental implants with good levels of bone-implant contact [22]. Seo et al. have demonstrated that SHEDs have the potential to repair critical-size calvarial defects in immunocompromised mice [23]. In models of mouse hind limb ischemia, local transplantation of side population (SP) cells from dental pulp brings about the successful engraftment and vasculogenesis [24].

Besides their potential applications in treating systemic diseases, DPSCs have been extensively explored in the area of tooth regeneration and are thought to be the putative candidate for dental tissue engineering, due to: (a) the easy surgical access to the collection site and very low morbidity after the extraction of dental pulp [1, 2, 12, 25, 26]; (b) autologous DPSCs can be efficiently isolated and amplified from an impacted molar or exfoliated deciduous tooth [1, 2, 25, 26]; (c) DPSCs can generate much more typical dentin tissues within a short period than non-dental stem cells, which makes them more competent in making a bio-tooth [9]; (d) the amount of newly formed dentin by ex vivo

Table 1 Stem cell types in dental pulps [1–3, 12, 21, 23, 25, 28, 35, 72, 78, 95, 97]

Properties	DPSCs	SCAPs	SHEDs
Location	Permanent tooth pulp	Apical papilla of developing root	Exfoliated deciduous tooth pulp
Specific markers	ND	ND	ND
Proliferation rate	Moderate	Higher	Higher
Heterogeneity	Yes	Yes	Yes
Multi-potentiality	Odontoblast, osteoblast, chondrocyte, myocyte, neurocyte, adipocyte, corneal epithelial cell, melanoma cell, iPS	Odontoblast, osteoblast, neurocyte, adipocyte, iPS	Odontoblast, osteoblast, chondrocyte, myocyte, neurocyte, adipocyte, iPS
Potential contributions to systematic diseases	Bone regeneration, central nervous degeneration, liver fibosis, myocardial infarction, corneal reconstruction	Bone regeneration	Bone regeneration
Potential contributions to bio-tooth	Dentin, pulp	Dentin, pulp, root	Dentin, pulp

ND not determined; *iPS* induced pluripotent stem cells

DPSCs far exceeds the overall dentin matrix during the entire lifetime synthesized by in situ odontoblasts [1, 12]; (e) DPSCs can be safely cryopreserved and recombined with many scaffolds [1, 12, 27–30]; (f) DPSCs seem to possess immuno-privilege and anti-inflammatory abilities favorable for the allotransplantation experiments [31, 32].

This review will focus on several important issues including the origin and isolation of these stem cells, the odontoblastic differentiation, and the potential applications of DPSCs in the making of a bio-tooth.

Localization and Origin of DPSCs

Although DPSCs have been isolated from pulp tissues in different types of teeth including exfoliated primary incisors, permanent teeth, natal teeth, and even supernumerary teeth [1–3, 12, 33–35], the accurate niche where these stem cells originate remains unclear. In a traditional view, dental pulp consists of four layers, i.e. the odontoblastic zone, cell free zone, cell rich zone, and central zone. The cell-rich zone is richly populated with stem/progenitor cells that display plasticity and multipotent differentiation capacity, and serves as a reservoir for the replacement of destroyed odontoblasts.

Shi et al. have demonstrated that the expression of stem cell markers (CD146 and STRO-1) in the dental pulp is restricted to blood vessel walls, but absent in the surrounding fibrous tissue, odontoblast layer, and perineurium of the nerve, indicating that DPSCs are localized in the perivascular region of pulp tissue [10]. STRO-1 positive DPSCs express the vascular antigens including von Willebrand factor, CD146, α-smooth muscle actin, and a pericyte-associated antigen (3G5), suggesting that these stem cells may be of a vascular origin and their histological location may be relevant to the microvascular system in the dental pulp [10].

The cDNA microarray analysis demonstrates that human DPSCs share the similar gene expression profile with bone marrow mesenchymal stem cells (BMMSCs). Both can express a variety of markers associated with endothelium, bone, and fibroblasts [36], implying that these stem cells may originate from bone marrow.

From the viewpoint of developmental biology, cranial neural crest (CNC) gives birth to most oral and dental tissues including dental pulps [37–39]. Thus, it is reasonable to assume that DPSCs are derived from CNC ectomesenchyme [40]. Sasaki et al. have demonstrated that DPSCs contain primitive stem cell subpopulations of neural crest origin, including Nestin$^+$ precursor cells, Tuj1$^+$ neuron cells, and S100$^+$ glial cells [41]. These stem cells can generate the neurospheres under the serum-free culture conditions. Our study has shown that DPSCs express several neural crest-related markers (S-100, nestin, CD57, CD271, glial fibrillary acidic protein) and share many similarities with cranial neural crest stem cells (CNCSCs) on the aspects of immunophenotypes and other biological behaviors (unpublished data). Following the initiation and maturation of tooth germ, DPSCs are thought to experience different developmental stages, i.e. odontogenic ectomesenchymal stem cells, dental papilla stem cells, dental pulp stem cells, dental pulp precursor cells, pre-odontoblasts and terminally differentiated odontoblasts.

Isolation Approaches of DPSCs

So far, no stable model has been set up to isolate and purify DPSCs for the lack of specific cell surface markers. The identification of DPSCs mainly depends on the biological characteristics, including small cell volume, vigorous proliferative ability, potent clonogenicity, self-renewal, and multi-differentiation potential [1, 12, 14]. Various methods have been developed to isolate stem cells from dental pulp, below are the conventional ones.

Size-sieved Isolation

Several studies have demonstrated that small size represents one of the important characteristics of adult stem cells [42–44]. These small diameter cells have greater viability, proliferative capacity and regenerative capability than those larger ones. Size-sieved stem cells derived from bone marrow can differentiate into bone, fat, cartilage, and neural cells [43]. During the odontoblastic differentiation of DPSCs, the cell volume will be significantly increased with a long cellular process, implicating that undifferentiated DPSCs are small diameter cell populations. Figure 1 shows the size-sieved protocol for the isolation of stem cells. Briefly, the whole dental pulp tissue is gently separated from dental hard tissues and digested in a solution of 3% collagenase type I for 1 h at 37°C. The single cell suspension is filtered through a 20 μm strainer to remove those larger differentiated cells. Then, these smaller cells are seeded onto a 3 μm porous sieve to collect the larger cells. Those cells on the upper plate surface of 3 μm sieve with a diameter between 3 μm and 20 μm are obtained for further culture and amplification. Based on this approach, small-sized cell populations containing a high percent of stem cells can be isolated from the single-cell suspension, and thus DPSCs can be preliminary purified.

Stem Cell Colony Cultivation

Colony culture is the classical method to purify stem cell from the mixture of heterogeneous cells. Colony culture can

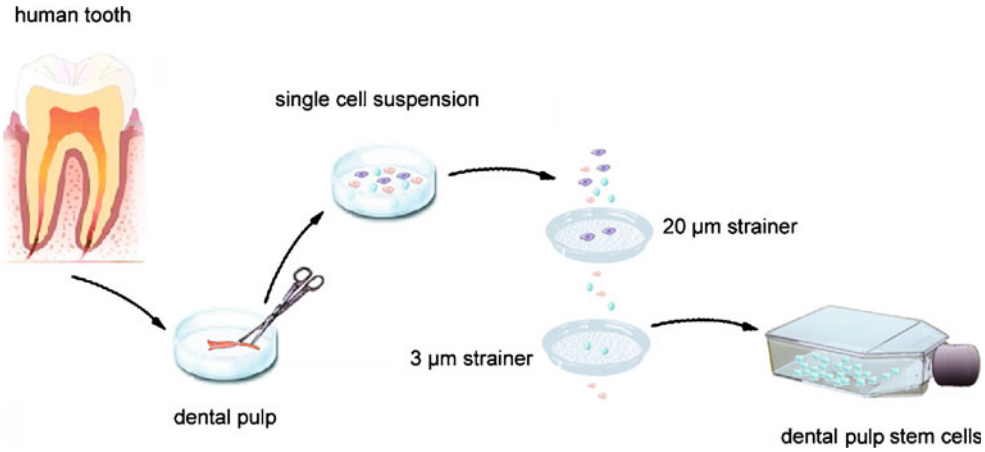

Fig. 1 Schematic diagram of size-sieved isolation. Dental pulps are isolated from freshly extracted teeth and digested with 0.3% type I collagenase to prepare the single cell suspension. The single cell suspension is filtered through a 20 μm strainer to obtain smaller cells. The single cell suspension containing smaller cells is loaded onto a 3 μm porous sieve to collect relatively larger cells on the upper plate surface. Thus, dental pulp stem cells (DPSCs) can be preliminarily purified

be initiated from a disaggregated cell suspension which is made directly from tissues or subcultured cells. Human DPSCs from a single cell colony have been extensively studied, manifesting the self-renewal capability and multipotent differentiation [1, 12]. Figure 2 shows the typical protocol for the isolation of DPSCs by colony culture. Firstly, the dental pulp tissue is digested to prepare the single cell suspension. Then, the cell suspension is diluted to 100 cells/mL and the cells in the suspension are placed into 96-well plates by 0.1 mL/well. The plates are pre-seeded with feeder cells which can provide the sufficient cell density, nutrition, growth factors, and extracellular matrix necessary for the colony formation. After in vitro incubation at 37°C in 5% CO_2 for 1–2 weeks, the single colony cluster containing 50 or more cells is digested and amplified for further experiments.

Stem cell colonies, particularly those initiated directly from tissues, are often different on the aspects of colony morphology, size and cell density. These differences between colonies are associated with the cell types, cell–cell interactions, differentiation potential, cell passages, medium composition, feeder cell types, and culture conditions. In addition, cell colony cultivation from one single cell is time consuming due to limited cell growth speed.

Magnetic Activated Cell Sorting

Magnetic activated cell sorting (MACS) is an immunomagnetic method used for the separation of stem cell populations based on their surface antigens (CD271, STRO-1, CD34, CD45, and c-Kit). This approach has been widely used for the isolation of many different cell types, including lymphocytes, dendritic cells, fetal cells from maternal blood, granulocytes, megakaryocytic cells, natural killer cells, T cells, tumor cells, epithelial cells, and hematopoietic stem cell [45]. Figure 3 shows the typical MACS protocol for the isolation of DPSCs. Firstly, dental pulp is isolated and single cell suspension is prepared. Secondly, the suspension is incubated in the test tube with primary monoclonal antibody against specific membrane surface molecule and subsequently with immunomagnetic beads. These magnetic beads are pre-coated with monoclonal secondary antibody necessary for searching and binding to target cells. Then, the mixture of cells and beads is placed in the magnetic particle concentrator, and cells specifically bound to beads will attach to the test-tube wall in the magnetic field. The bead-free cells in the supernatant are discarded. Finally, target cells bound to beads are

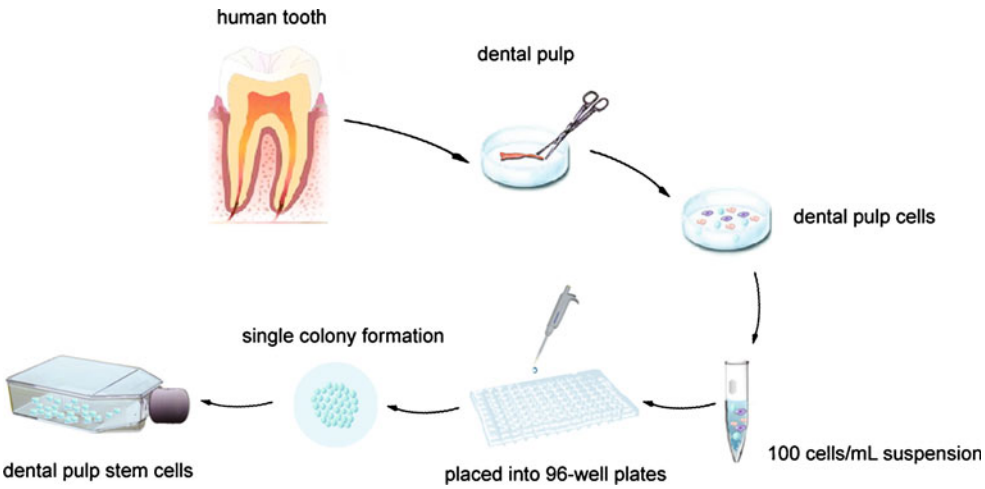

Fig. 2 Schematic diagram of stem cell colony cultivation. Dental pulps are digested to obtain the single cell suspension. The single cell suspension is diluted to 100 cells/mL, placed into 96-well plates by 0.1 mL/well, and subsequently cultured for 1–2 weeks to observe the colony formation. Each colony will be investigated to obtain DPSCs according to the self-renewing and multipotent differentiation capacity

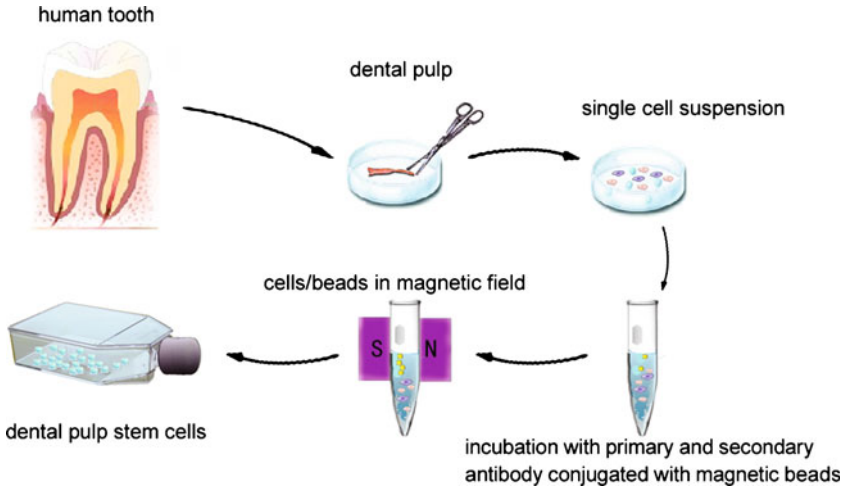

Fig. 3 Schematic diagram of magnetic activated cell sorting. Dental pulps are digested into single cell suspension. The suspension is incubated in the tube with primary monoclonal antibody against stem cell surface marker (e.g. STRO-1), and then incubated with the immunomagnetic beads-conjugated secondary antibody. The cell/bead mixture is then put into the magnetic field to collect those cells attached to the tube wall. Then, attached cells are resuspended and subcultured to obtain DPSCs

selectively collected when passing through the magnetic field. The resultant cells are re-suspended and cultured for further experiments. To date, several subpopulations including STRO-1$^+$ and CD271$^+$ DPSCs have been successfully isolated by MACS method.

Generally speaking, MACS is technically simple, inexpensive and capable of handling large numbers of cells, which provides a practical avenue for the lineage-specific selection of stem cell progenies. However, the degree of stem cell purity is not much high, that may in turn compromise the efficiency of stem cells used in further investigations.

Fluorescence Activated Cell Sorting

The fluorescence activated cell sorting (FACS) is a convenient and efficient method that can effectively isolate stem cells from cell suspension based on the cell size and fluorescence. Cells are usually stained with one or more fluorescent dyes specific to targeted cell components. Fluorescence of each cell is quantified as it rapidly transects the laser beam and fluorescence intensity provides the basis for separation of cell subpopulations.

Using this method, STRO-1 selected DPSCs can perform the odontblastic differentiation and hard tissue formation under certain conditions [46–48]. c-kit$^+$/CD34$^+$/STRO-1$^+$ DPSCs can differentiate into several kinds of ectomesenchyme-derived mature cells [49–51]. Furthermore, Hoechst 33342-sorted SP cells from dental pulp have the typical features of stem cells, including the self-renewal and multipotent differentiation potential [24, 52].

However, FACS is a highly sophisticated technique which requires expensive equipment and highly-skilled personnel. FACS-sorted cells often undergo physiological stress and decreased viability. Moreover, this method is not appropriate for processing bulk quantities of cells that would almost certainly be required for therapeutic applications [53]. Instead, FACS seems to be more suitable for the research or diagnostic analysis of relatively small cell populations.

Differentiation of DPSCs into Odontoblasts

Cell differentiation refers to the progressive specialization of cell morphology and function that leads to the formation of specialized cells, tissues, and organs, accompanying with the differential expression of specific genes. It usually brings about the variations in cell volume, appearance of new surface markers, modifications of enzyme activity, and even the changes in the cellular protein composition.

DPSCs can be reprogrammed into multiple cell lineages (Table 1) and even induced pluripotent stem cells (iPS) [1, 2, 10, 12, 14, 27, 50, 54–57]. The differentiation of DPSCs to a specific cell lineage is mainly determined by the components of local microenvironment, including growth factors, receptor molecules, signaling molecules, transcription factors and extracellular matrix proteins [29]. He et al. have proved that the synergy of Notch-Delta1 signaling and differentiation-inducing factors (DIFs) can drive human DPSCs into odontoblast lineage [58]. Almushayt et al. [59] have demonstrated that dentin matrix protein 1 (DMP1, a non-collagen extracellular matrix protein extract from dentin) can significantly promote the odontoblastic differentiation of DPSCs and the formation of reparative dentin over the exposed pulp tissue. During the odontoblastic differentiation, core binding factor alpha1 (CBFA1) can upregulate *DMP1* gene expression and contribute to the spatial-temporal expression pattern of DMP1 [60]. Additionally, DPSCs can be induced into odontoblast lineage when treated with transforming growth factor β1 (TGFβ1) alone or in combination with fibroblast growth factor

(FGF2) [61]. The simvastatin (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor) and DMP1-derived peptide also have the ability to trigger the differentiation of DPSCs into odontoblasts [62, 63]. Some other molecules including EphB/ephrin-B and bone morphogenetic proteins (BMPs) also play a pivotal role during the perivascular migration towards the dentin surface and odontoblastic differentiation of DPSCs [64, 65].

Although many single-factors can induce the differentiation of DPSCs, in situ microenvironment of DPSCs is much complicated, which involving many matrix components, mineral ions, and growth factors. The odontoblastic differentiation induced by only one or two growth factors may cause undesirable biological changes in differentiated DPSCs. Since dissociated tooth germ cells keep all the morphogenetic information necessary for the bio-tooth development, conditioned medium extracted from tooth germ cells has been used to mimic the microenvironment of growth factors which can effectively initiate the odontoblastic differentiation of DPSCs [29, 66, 67].

Some physical factors (e.g. zero gravity, stress, and trauma) also impact the differentiation of DPSCs. In clinical practice, the pulp calcification caused by abnormal chewing force may attribute to the functional modifications in DPSCs. When in vitro DPSCs are subjected to the dynamic hydrostatic pressure (HSP), their adhesion capacity and viability are significantly compromised, while their mineralization capability and odontoblastic differentiation potential are markedly enhanced [68].

When exogenous *Gdf-11* gene is imported into DPSCs by electrical transfection method, it can trigger the odontoblastic differentiation of these stem cells and bring about the formation of dentin-like structures [69]. The genes related to odonto/osteo-blastic differentiation (*Alp*, *Ocn*, *Col-1*, *Bsp*, and *Dspp*) can be up-regulated when *Bmp-2* gene is transferred into STRO-1^+ DPSCs [47]. Moreover, iPS can be made by genetic engineering approaches when four genes (*c-Myc/Klf4/Oct4/Sox2* or *Lin28/Nanog/Oct4/Sox2*) are introduced into DPSCs [57]. These dental iPS cells have multiple functions of embryonic stem cells, such as generating embryoid bodies in vitro and teratomas in vivo, and can replace embryonic stem cells to manufacture various tissues and organs.

Regeneration of Dentin-pulp Complex by DPSCs

In the process of tooth development, dental pulp and dentin are derived from the embryonic dental papilla. Histologically, dentin lies outside of dental pulp, and they closely link to each other. Functionally, dental pulp cells can regenerate dentin, and provide it with oxygen, nutrition, and innervation, whereas the hard dentin can protect soft dental pulp tissue. They together maintain the integrity of tooth shape and function. Any physiological or pathological reaction occurring at one part, such as abrasion, caries, and cavity preparation, will affect the other. Both of them act as a dentin-pulp complex and simultaneously participate in various biological activities of tooth.

Many studies have shown that DPSCs play a paramount role in the dentin-pulp tissue regeneration [1, 2, 9, 12, 29, 69–74]. Gronthos et al. have recombined human DPSCs with hydroxyapatite-tricalcium phosphate (HA-TCP) ceramic powder, and transplanted them subcutaneously into immunocompromised mice. The recovered tissues contain the typical dentin structures surrounded by odontoblast-like cells with long cytoplasmic processes deeply into the mineralized matrix. Some dental pulp-like structures containing blood vessels can be observed around the dentin matrix [1, 12, 29, 69]. Similar dentin formation can be detected in in vivo SHEDs recombined with HA-TCP scaffolds [2]. Furthermore, DPSCs can produce dentin-pulp-like complex in the scaffold and reparative dentin-like structure on the surface of dentin slice [71, 74].

Our previous study has demonstrated that dental mesenchymal cell pellets at the late developmental stages can reexhibit the dentinogenesis in the absence of epithelial-mesenchymal interactions and form the typical dentin-pulp-like complex containing distinctive odontoblasts, predentin, dentin and dentinal tubules [75]. After the induction in tooth germ cell-conditioned medium, transplanted DPSCs pellets in the renal capsules can develop into regular dentin-pulp-like complex [29]. These findings suggest that dental mesenchymal cells including stem cells can perform the dentinogenesis independently without the existence of dental epithelial components. Since DPSCs have the potent dentinogenic ability, they can be used for the vital pulp therapy. When DPSCs are transplanted alone or in combination with BMP2 in the pulp cavity, these stem cells can significantly promote the repair and reconstruction of dentin-pulp-like complex [76]. Prescott et al. [77] have placed the triad of DPSCs, a collagen scaffold, and dentin matrix protein 1 (DMP1) in the simulated perforation sites in dentin slices, and then transplanted the recombination subcutaneously into the nude mice. After 6 weeks of incubation, well-organized pulp-like tissues can be detected in the perforation site. Cordeiro et al. [78] have demonstrated that SHEDs/scaffold recombinations prepared within human tooth slices also have the potential to form dental pulp-like structures. Huang et al. [72] have reported that dentin-pulp-like complex with well-established vascularity can be regenerated de novo in emptied root canal space by either DPSCs or SHEDs. These researches provide a novel approach for future pulp tissue preservation and an innovative choice of the biological treatment for endodontic diseases.

Reconstruction of Bio-tooth from DPSCs

Tooth loss is a common and frequently occurring disease in the aging populations that adversely affects the masticatory efficiency, language function, facial esthetics, and psychological health. In the developed countries, an estimated 7% of people have lost one or more teeth by age 17. After age 50, an average of 12 teeth have been lost [79]. World Health Organization (WHO) databanks demonstrate that dental caries is still prevalent in most countries worldwide (100% incidence in some populations); severe periodontal diseases which may result in tooth loss are estimated to affect 5–20% of most adult populations, and the incidence of complete edentulism has been estimated between 7% and 69% internationally [80, 81]. To treat these missing teeth, current approaches mainly focus on the artificial materials or non-biological implants that can unavoidably reduce the quality of life due to their limited physiological functions and sometimes elicit an immune rejection.

Bio-tooth is thought to be a kind of biological tooth that can be re-integrated into the jaw and perform the normal functions of a natural tooth including the regenerative ability in case of injury. Using the principle of epithelial-mesenchymal interactions to guide the tooth regeneration has become a common strategy in dental tissue engineering. Many studies have demonstrated that the bio-tooth can be reconstructed by dental cells recombined with or without scaffolds, by pre/post-natal dental cells, and even by non-dental cells [9, 29, 66, 67, 70, 82–87]. Nakao et al. have demonstrated that bioengineered incisor tooth germs can be reconstituted using completely dissociated dental epithelial and mesenchymal cells in a three-dimensional collagen gel [88]. These bioengineered tooth germs can replicate the embryonic tooth organogenesis and develop into the whole incisor in vitro or in the dental alveolus of adult mice [88]. Furthermore, Ikeda et al. [89] have proved that these bio-teeth in the alveolar bone can perform the functions of a natural tooth including the eruption, occlusion and mastication, which highlights a new exciting prospect of bio-teeth in future clinical applications.

Sonoyama et al. have proposed the stem cell-based bio-root regeneration by integrating several approaches together including stem cell techniques, biomaterials, and crown restoration [3]. The recombinations between SCAPs/HA-TCP and periodontal ligament stem cells (PDLSCs) can bring about the formation of bio-root/periodontal complexes, which can support a porcelain crown to restore the normal masticatory and aesthetic functions [3]. Previous work has revealed that DPSCs pellet reassociated with adult rat apical bud cells can form a typical crown-like structure in vivo containing distinctive ameloblast layer, enamel, dentin, predentin and odontoblast layer [9]. This study has general implications in rebuilding a bio-tooth with adult dental stem cells. Further studies indicate that the proportion of epithelial and mesenchymal cells is much important to the regulation of normal crown morphogenesis [9, 70, 87]. However, no root-like structure has been observed during DPSCs-based tooth regeneration, due to the complex mechanism inherent in root development.

Recently, many types of cells including DPSCs, SHEDs, and SCAPs have been successfully reprogrammed into iPS cells which hold a great promise for regenerative medicine [57, 90–94]. These dental iPS cells express the marker genes that characterize embryonic stem cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers [57]. Our

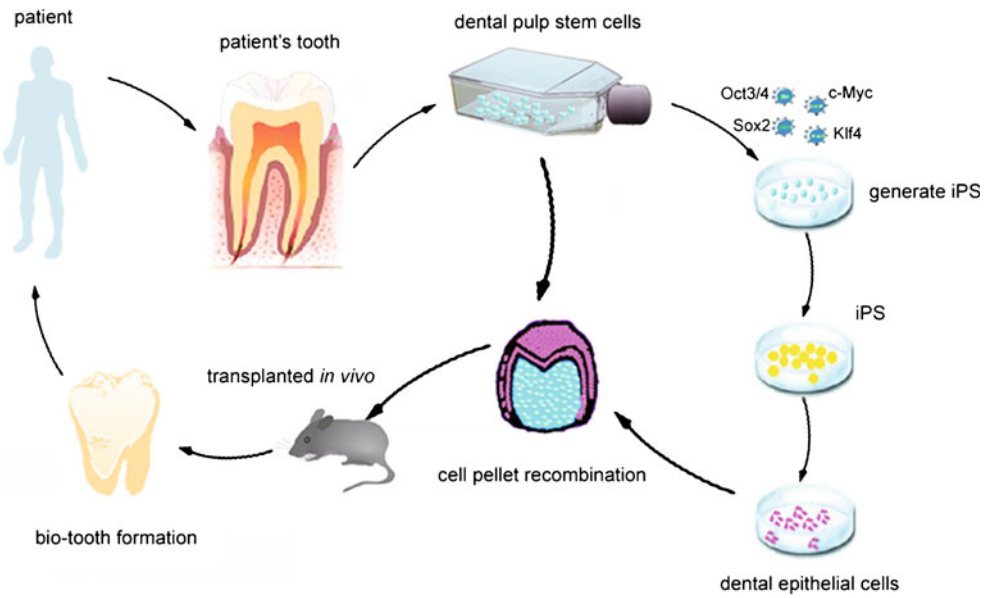

Fig. 4 Schematic diagram illustrating the making of a bio-tooth using dental iPS cells. Autologous DPSCs are isolated from the patient's own dental pulps. Pluripotent iPS cells are created by driving four genes (*c-Myc, Klf4,Oct3/4,and Sox2*) into DPSCs, which can be used to generate dental epithelial cells under suitable conditions. Then these iPS-derived dental epithelial cells are recombined with autologous DPSCs pellet and transplanted in vivo to make a bio-tooth. After the temporary incubation in vivo, bio-tooth can be transplanted into the patient's jaws to treat tooth loss

unpublished data further reveal that the marker genes of ameloblast lineages (ameloblastin and amelogenin) are significantly up-regulated in DPSCs-derived iPS cells, as indicated by real-time RT-PCR analyses. Thus, whether we can use these dental iPS cells to make a bio-tooth is still full of uncertainty and challenges. Figure 4 is the feasible strategy for building a bio-tooth using dental iPS cells, in which all dental cells are autologous. Firstly, autologous DPSCs are isolated from the patient's own dental pulp and amplified in vitro. Secondly, iPS cells are generated by driving four genes (*c-Myc/Klf4/Oct4/Sox2*) into DPSCs [57]. These dental iPS cells can give birth to dental epithelial cells under suitable conditions because of their pluripotent properties. Then, these iPS-derived dental epithelial cells are reassociated with autologous DPSCs and subsequently transplanted in vivo for temporary incubation to produce a bio-tooth [9]. Finally, bio-tooth germ or whole bio-tooth can be transplanted into the patient's jaws for continuous growth and eruption.

Perspectives

Although DPSCs will play a fundamental role in various human tissue regenerations in the future, the main commitment of these dental cells is located in the area of tooth regeneration, where they can find an immediate application. Autologous DPSCs collected from dental pulps of permanent teeth can be used in situ for different therapeutic purposes. Future strategies will no doubt concentrate on the differentiation mechanism of these dental stem cells, optimization of bio-scaffolds, and exploration of odontogenic microenvironment necessary for the odontoblastic differentiation.

Clearly, recent recognitions of dental stem cells and their role in making a bio-tooth provide a substantial basis upon which we can begin to explore their therapeutic potential at the preclinical level. However, making a bio-tooth with masticatory function and supportive tissues from dental stem cells may be much more complicated than expected. Key techniques must be developed to reproduce the highly specialized arrangements of dental stem cells that constitute a bio-tooth. Several issues involving in the making of a stem cell-mediated bio-tooth must be solved, including identification and 'stemness' maintenance of stem cells, dental morphogenesis, tooth type determination, odontogenic signal cascades, odontogenic epithelium availability, controllable bio-tooth growth and eruption, pulp revascularization and neural regeneration, and host-graft immunorejection in the jaws [70, 87, 95, 96]. Although there are many technical barriers to overcome, stem cell-based approaches to tooth reconstruction will, of necessity, provide an unpredictable applicative opportunity to treat tooth loss and other dental diseases.

Acknowledgements This work was supported by Nature Science Foundation of Jiangsu Province, China (No. BK2009346).

Conflict of interest The authors have declared that there is no conflict of interests embedded in this manuscript.

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