Japanese Dental Science Review (2015) 51, 75—83

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Review Article

Ability of stem and progenitor cells in the dental pulp to form hard tissue

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Received 12 November 2014; received in revised form 24 February 2015; accepted 13 March 2015

KEYWORDS
Dental pulp;
Odontoblast;
Stem cells;
Dentin formation;
Differentiation;
Pulp biology;
Oral anatomy;
Oral biology

Summary Dental pulp has an important ability to form mineralized hard tissue in response to a variety of external stimuli. The formation of mineralized tissue within the pulp cavity has been widely examined in both clinical and animal studies. Despite these studies focusing on the phenomena of reparative dentin and dentin bridge formation, the mechanisms of their induction remain unknown. Recently, several morphological studies revealed that the source of cells for hard tissue formation is the dental pulp itself, even after pulp injury. This finding indicates that the dental pulp tissue contains undifferentiated cells participating in dentin and pulp regeneration. Additionally, stem and progenitor cells isolated from the dental pulp were found to differentiate into odontoblasts as well as osteoblasts. This review presents current evidences for the multipotent ability of dental pulp cells and their usefulness in tissue engineering applications as a cell resource.

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http://dx.doi.org/10.1016/j.jdsr.2015.03.002

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1. Introduction

Dental pulp is a loose connective tissue surrounded by dentin. Because hard tissue formation by odontoblasts and pulp cells is an important protective response to external stimuli, every effort must be made to maintain their vitality and function. Four distinct zones are histologically distinguishable in the dental pulp. The outermost layer of the pulp is termed the odontoblast layer, which is composed of odontoblasts forming dentin under normal physiological conditions. Beneath this layer, a cell-free zone (zone of Weil) is observed specifically in the coronal pulp, and it connects with a cell-rich zone characterized by high cell density. These cell-free and cell-rich zones are collectively referred to as the subodontoblastic layer [1]. Besides the surface cell layers, the central region of the pulp is populated by the major vessels and nerves as well as by dental pulp cells and extracellular matrix. The dental pulp tissue contains several types of cells such as odontoblasts, fibroblasts, macrophages, dendritic cells, as well as undifferentiated mesenchymal cells that regulate the homeostatic function of the dentin—pulp complex [2].

Recent progress in identifying stem and progenitor cells from adult tissues suggests their potential application for clinical use in some fields [3,4]. Mesenchymal stem cells have been recognized in many organs and tissues such as skeletal muscle and central nervous system [5,6], as well as classically in hematopoietic lineage, skin, and gut [7—9]. In addition, the existence of stem cells within the dental pulp has been reported in the case of human permanent [10] and deciduous [11] teeth. These dental pulp stem cells show self-renewal ability and multilineage potential [12,13]. The pulp tissue also contains a wide variety of undifferentiated cells that can differentiate into odontoblast-like cells. Many morphological studies have suggested that the dental pulp is capable of forming hard tissues including dentin and bone [14,15]. The mechanisms of pulp calcification have also been analyzed by use of *in vivo* experimental techniques such as tooth [16] and pulp [17] transplantation.

This review focuses on pulp function regarding the formation of hard tissue. Additionally, we introduce recent findings on a cell population having hard tissue-forming ability, and thereafter discuss the potential of pulp stem cells for regenerative therapy.

2. Pulp calcification

The formation of mineralized tissue within the pulp cavity has been widely examined in both clinical and experimental animal studies. Many reports have described reparative dentin and dentin bridge formation following injuries such as dental caries [18,19], cavity preparation [20—25], and direct pulp capping [26—30]. Of interest, bone-like tissues are found after tooth replantation [31—33], traumatic injury [34], and laser irradiation [35—37], in addition to dentin formation. During the reparative process in the injured pulp, primary odontoblasts lost as a result of extensive damage are replaced by newly differentiated hard tissue-forming cells secreting a dentin- or bone-like matrix [38]. This process consists of the sequential steps of proliferation, migration, and differentiation of stem and progenitor cells, along with reinnervation and revascularization [39].

The cell origin of the hard tissue-forming cells after pulp injury is still controversial. Several possibilities can be proposed to explain the origin of cells involved in pulp regeneration. One is that bone marrow-derived mesenchymal cells participate in osteoblast-like cell differentiation. Such undifferentiated cells might be supplied to the injured pulp *via* the bloodstream and then differentiate into hard tissue-forming cells [40,41]. A second possibility is that stem and/or progenitor cells in the dental pulp itself differentiate into odontoblast- or osteoblast-like cells and form hard tissue. This possibility is supported by earlier reports indicating that undifferentiated mesenchymal cells exist within the dental pulp [10,11]. The majority of these stem cells are found at the periphery of blood vessels in the central region of the pulp [42,43]. The cells in the subodontoblastic cell-rich layer have also been shown to have high mineralization ability and form a bone-like matrix *in vivo* [44]. Moreover, the periodontal ligament (PDL) is thought to be a source of hard tissue-forming cells. PDL cells possess high alkaline phosphatase activity [45] and produce bone matrix proteins [46] and mineralized nodules [47] under osteoinductive culture conditions. Indeed, they extensively migrate after transplantation [48,49]. In this context, several morphological studies demonstrate that hard tissue formation in dental pulp cells is mainly caused by the pulp cells themselves, and merely formed by mesenchymal cells that entered from outside (Table 1).

Several reports have described hard-tissue formation in the pulp cavity of rat molar after tooth transplantation into subcutaneous tissue [14,50,51]. Because pulp calcification can be investigated without inflammation due to infection, this study model is very useful and reproducible. After transplantation, the coronal pulp cavity becomes necrotic at the early period, and then three distinct types of mineralized hard tissue are formed in the pulp. Cell-rich hard tissue is formed at the root apex and is immunonegative for dentin sialoprotein (DSP), which is a marker of the dentin matrix [52,53]. This tissue resembles bone from both histological and immunohistochemical perspectives. Additionally,

Table 1 Experimental animal models of pulp calcification.

Implant	Recipient	Matrix property	Origin of forming cells	References
Whole tooth	Hypodermis	Dentin and bone	Pulp	[14,16]
Whole tooth	Tooth socket	Dentin/bone	Pulp/pulp and recipient	[31—33,41]
Tooth crown	Submucosa	Dentin/bone	Pulp/pulp and recipient	[15,40,55]
Pulp	Hypodermis	Bone	Pulp	[17,61—63]

DSP-positive dentin-like matrix present adjacent to the dentin in the root canals. Furthermore, formation of the bone-like tissue occurs apart from the dentin in the coronal pulp cavity [14]. This bone-like tissue contains bone marrow-like tissue, whereas there is no evidence of vascularization in the apical cell-rich hard tissue [16]. Next, to analyze the origin of these hard tissue-forming cells that appear in the pulp cavity, green fluorescent protein (GFP)-labeled teeth were transplanted into the hypodermis of GFP-negative host rats. This xenograft tooth transplantation model showed that GFP immunoreactivity is detectable in both odontoblast- and osteoblast-like cells, indicating that the dental pulp contains progenitors of hard tissue-forming cells. Therefore, undifferentiated pulp cells can differentiate into not only odontoblast-like cells but also osteoblast-like ones in severely injured pulp and reestablish pulp vitality.

In the case of tooth replantation, dentin- or bone-like tissue is present in the pulp cavity [31—33]. The xenograft model of rat tooth replantation using GFP-positive tooth and GFP-negative tooth sockets revealed that cells lining the bone-like tissue are either immunopositive or immunonegative for GFP [41]. This finding indicates that the bone-like tissue formation in the replanted teeth is accomplished by both host and donor cells. As noted above, pulp cells have the ability to differentiate into osteoblasts [16]. GFP-negative cells in the bone-like tissue might arrive from the recipient socket *via* the bloodstream, because the periodontium contains multipotential stem cells [54]. On the other hand, all cells lining the dentin-like matrix were immunopositive for GFP. All GFP-positive cells in the dentin-like tissue imply that mesenchymal cells of the recipient socket could not differentiate into odontoblast-like cells. If the nutrition recovery begins early and maintains the cellular activity of the odontoblasts, reparative dentin or dentin-like matrix is formed. Otherwise, undifferentiated pulp cells might differentiate into odontoblast-like cells.

Since the coronal region of the pulp cavity becomes necrotic in the models of tooth transplantation and replantation, it is difficult to determine whether the coronal pulp contains hard tissue-forming cells. Ohshima et al. [15,40,55] performed experiments in which mouse molars resected at the root and pulp floor were transplanted into the submucosa in the oral cavity. This model demonstrated proliferation of coronal pulp cells and formation of both dentin- and bone-like matrices. Their experiments using LacZ-transgenic mice showed that coronal pulp cells form the dentin-like matrix. On the other hand, cells forming bone-like matrix are of either pulp or recipient tissue origin [40]. These findings suggest that the coronal pulp also could harbor undifferentiated recipient cells having the ability to form hard tissue.

These above reports evaluated the pulp calcification in the presence of dentin matrix around the pulp tissue. Dentin contains several growth factors such as bone morphogenetic proteins (BMPs) and transforming growth factors [56,57]. These growth factors promote odontoblast differentiation and calcified matrix formation *in vivo* [58,59] and *in vitro* [60]. Thus, it is unclear whether pulp calcification is caused by the pulp cells themselves or induced by growth factors in the dentin. Then, for investigation of the mechanism of pulp calcification without the effects of growth factors in the dentin, isolated rat incisor pulps were transplanted into various connective tissues, including subcutaneous tissue, kidney capsule or anterior chamber of the eye. After transplantation, these isolated pulps induce bone-like calcified tissues [61—63]. Transplantation of GFP-labeled pulp into wild-type rat hypodermis also showed that these formative cells are derived from the transplant [17]. This model revealed that pulp cells are able to form mineralized hard tissue in the absence of dentinal growth factors, although the morphology of the tissue formed does not resemble that of dentin. Epithelial cell signals and/or dentin-derived growth factors might be required for the formation of dentin matrix. In contrast, an earlier experiment involving subcutaneously transplanted rabbit muscle showed the formation of cartilage [64]. Perichondrium and periosteum transplants also appeared to induce cartilage and bone, respectively [62]. Therefore, the origin of the transplanted tissue might determine the type of hard tissue in concert with the surrounding environment and the nutrition supply.

3. Stem and progenitor cells in the dental pulp

Although the dental pulp undergoes calcification following various stimuli, the precise cell population that has the ability to form hard tissue in the pulp remains unknown. These hard tissue-forming cells in the dental pulp are thought to be localized either in the perivascular region or in the subodontoblastic cell-rich layer [65,66]. The dental pulp contains undifferentiated cells expressing stem and progenitor cell markers such as STRO-1, ABCG2, CD90, α-smooth muscle actin, and Bmi1 [12,67—70]. Among them, STRO-1 and ABCG2-positive cells are evident in the perivascular region under normal physiological conditions [71,72]; on the other hand, the majority of cells in the subodontoblastic layer express CD90 [44]. Additionally, some pulp cells expressing Bmi1 and/or α-smooth muscle actin appear after pulp injury [16,73]. Considering regenerative therapy, it is

critical to select and to isolate undifferentiated cells that can efficiently differentiate into the target cells.

3.1. Dental pulp stem cells (DPSCs)

In 2000, Gronthos et al. [10] reported that the dental pulp of human permanent teeth contains mesenchymal stem cells. They obtained single-cell suspensions of dental pulp and then isolated DPSCs and found that the proliferation ability of these DPSCs is higher than that of bone marrow-derived mesenchymal cells. These DPSCs also show multipotency, being able to differentiate into osteoblasts, neuronal cells, and adipocytes [13,74]. After transplantation of DPSCs with hydroxyapatite/tricalcium phosphate powder into the hypodermis of immunocompromised mice, these cells generated a dentin—pulp complex composed of odontoblasts and pulp cells [75,76]. Pulp stem cells can be isolated from human deciduous teeth by the same technique, and referred to as stem cells from human exfoliated deciduous teeth (SHED) [11,77].

3.2. DPSCs mobilized by G-CSF (MDPCs)

Although shown to have stem-cell properties, DPSCs are thought to be a heterogeneous population of cells. Granulocyte-colony stimulating factor (G-CSF) is known to have the ability to mobilize some stem cells [78]. Thus, there have been attempts to isolate DPSC subsets by using a transwell culture system on the basis of a migratory response to G-CSF. MDPCs possess multipotency as well as a high proliferation rate and migration ability compared with the DPSC population. Transplantation of MDPCs also induces pulp- and odontoblast-like cell differentiation [79—82]. Since this method can yield a stem cell population at high efficiency, the isolation of dental pulp stem cells responding to G-CSF may be useful for clinical application of dentin and pulp regeneration.

3.3. Side population (SP) cells

Side population (SP) cells appear as a tail of dimly stained cells by Hoechst 33342, a DNA-binding fluorescent dye. Through a mechanism driven by a membrane transporter, the breast cancer resistance protein (BCRP1), SP cells are obtained by dual-wavelength flow cytometry [83]. SP cells have been found in several kinds of tissues, including dental pulp [65,66,84—86] and periodontal ligament [87,88], and shown to be a population rich in stem cells. Pulp SP cells show self-renewal potential with a long proliferative lifespan and multipotency, and they can differentiate into chondrocytes, adipocytes, and nerve cells *in vitro*. Following stimulation with BMP-2, pulp SP cells differentiate into odontoblasts expressing *dentin sialophosphoprotein* (*dspp*); and transplantation of these stimulated SP cells onto the surface of exposed pulp induces dentin bridge formation [85]. Since some BCRP1-positive cells, *i.e.*, SP cells, were detected in the perivascular region, pulp SP cells were further isolated by use of an endothelial progenitor cell and endothelial cell marker, CD31 [89]. In the pulp SP cell population, the $CD31^-$ fraction more highly expresses stem cell markers than does the $CD31^+$ fraction, and the former secretes angiogenic and neurotrophic factors. This fraction has high inductive ability for angiogenesis and neuralization and also shows multipotency. So, in more recent studies, a complete regeneration of pulp tissue was attempted by using $CD31^-$ SP cells after pulpectomy [90—92]. $CD31^-$ SP cells were inserted into a dog root canal with a collagen scaffold. After 14 days, pulp-like tissue is regenerated with functional blood vessels and nerves as well as a layer of odontoblast-like cells producing reparative dentin. This study group succeeded in inducing a complete pulp tissue in the same transplantation model by using $CD105^+$ cells isolated from pulp cells [93,94].

3.4. Label-retaining cells (LRCs)

Bromodeoxyuridine (BrdU) selectively binds DNA in proliferating cells, and this binding is robust and durable. A previous report showed that some skin keratinocytes that had incorporated BrdU retain this label even after several rounds of cell division [95]. Hence, if BrdU is introduced during embryogenesis, cells existing in the embryonic stage and/or experienced few cell divisions can be detected *via* BrdU immunostaining after birth. This method supposedly marks cells, termed label-retaining cells (LRCs), including both stem cells and transit amplifying cells. Cells of LRC fraction in the dental pulp are partially consistent with SP cells, and these cells express mesenchymal stem cell markers [65]. After tooth transplantation into the submucosa, LRCs proliferate and differentiate into odontoblast- or osteoblast-like cells [96—98]. Since LRCs are localized at the periphery of blood vessels in the central region of the pulp under normal conditions in the mature mouse tooth [65,99], this stem cell niche in the pulp is mainly associated with blood vessels. The maintenance of pulp LRCs may be essential for regeneration of odontoblast-like cells during pulp healing processes in the severely injured pulp.

3.5. Cells in the subodontoblastic layer

The cells of the subodontoblastic cell-rich layer in the dental pulp are considered to contain odontoblast progenitor cells because of their positional relationship with odontoblasts. Higher alkaline phosphatase (ALP) activity, which plays a pivotal role in tissue calcification, is observed in the subodontoblastic layer compared with that in the odontoblast layer [100,101]. Thus, in light of its higher ALP activity combined with its anatomical location, the subodontoblastic layer has been speculated to provide hard tissue-forming cells when odontoblasts are injured or their cellular activity is lost [20,102]. CD90, also known as Thy-1, is a cell-surface marker of some stem [103,104] and progenitor cells [105,106]; and this protein reveals the specific localization pattern of such dental pulp cells in the subodontoblastic cell-rich layer. The CD90-expressing fraction isolated from dental pulp highly expresses odontogenic markers. Additionally, cells of this fraction show immediate elevation of ALP activity under culture condition and form numerous mineralized matrix after transplantation into hypodermis (Fig. 1a and b) [44]. These results suggest that cells in the subodontoblastic layer maintain early odontoblastic characteristics

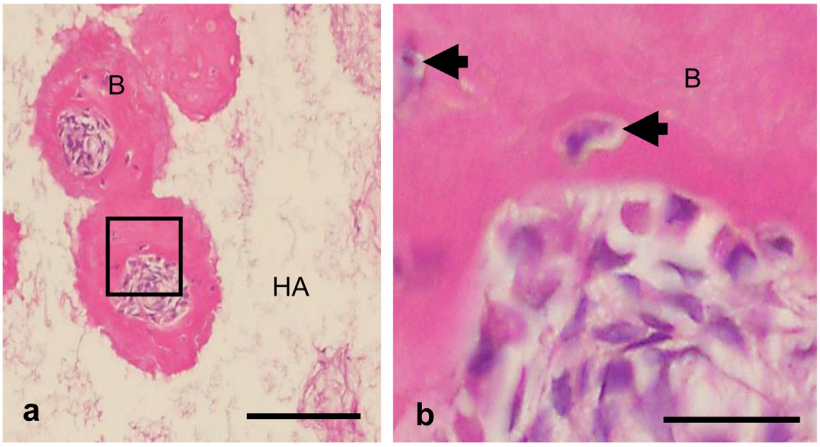

Figure 1 Transplantation of CD90-expressing cells on hydroxyapatite (HA) disk into subcutaneous tissue. (a) Newly-formed bone-like matrix (B) is found in some pores of the HA disk. (b) This matrix contains cells (arrows) and does not show any dentinal tubular structure. Scale bar: 150 μm (a), 30 μm (b).

and can immediately differentiate into hard tissue-forming cells in the case of odontoblastic injury. This subodontoblastic cell-rich layer might serve as a pool of odontoblast progenitors.

4. Inducing factor for pulp calcification

Although mesenchymal cells in the adult tooth scarcely form calcified tissue under normal conditions, the dental pulp shows intense mineralization activity after tooth extraction, such as under culture conditions [107—110], transplantation [14,16] or replantation [41,111]. In the normal dental pulp, ALP activity is detected only in odontoblast and subodontoblastic layers *in vivo*. The central part of the dental pulp hardly demonstrates this activity [100,101]. However, hard tissues are formed in most areas of the pulp cavity after pulp injury. The dental pulp has a dense nerve supply, and the most of the nerves are accompanied with blood vessels [112]. Many neuromodulatory molecules are expressed in the dental pulp [113,114]. Of these molecules, nerve growth factor, brain-derived neurotrophic factor [115], and neurotrophins [116,117] have been shown to participate in tooth mineralization. This neuromodulatory action is interrupted by tooth extraction, and nerve reconstruction might be delayed after tooth transplantation. Certainly, one can speculate that several key molecules are closely associated with pulp calcification. It is possible that neurological activity may be a candidate regulator of the differentiation of hard tissue-forming cells in the dental pulp. Further research regarding the mechanisms underlying cell differentiation of odontoblast-like cells is required.

5. Perspective

Cell replacement therapy using undifferentiated cells is considered to be one of the most effective methods for cell and tissue regeneration. It is possible to collect stem cells from dental pulps extracted as pulp extirpation treatment or from a nonfunctional third molar. For cell replacement therapy, a large number of stem cells would be required. Since stem cells in the dental pulp have higher proliferating ability than those in other tissues [10,11,85], the utilization of pulp cells may be practical for regeneration therapy. Therefore, pulp stem cells have been studied for potential application in the case of the regeneration of many tissues and organs.

Stem cells isolated from the dental pulp of human third molars differentiate into functional hepatocytes producing albumin after stimulation by appropriate growth factors *in vitro*; and transplantation of these cells into liver with hepatic inflammation prevents the progression of liver fibrosis and adipose degeneration [118]. Additionally, a revascularization technique has been tested by using pulp SP cells isolated based on CD31 for endothelial progenitor cells and endothelial cells [89] and on CD146 for endothelial cells and smooth muscle cells [119]; and it has revealed that $CD31^{-}/CD146^{-}$ SP cells express several angiogenic factors and form blood vessel-like structures in three-dimensional cultures. Transplantation of these cells into mouse hindlimb and brain under ischemic conditions promotes angiogenesis at this site [42,94,120], suggesting that some pulp cells have potential therapeutic application for ischemic disease. Furthermore, pulp stem cells demonstrate a high potential to serve as a resource for neuroprotection. When co-cultured with pulp stem cells, brain cells that had been exposed to various toxic factors could avoid cell death and apoptosis due to the secretion of anti-oxidants from these stem cells [121]. DPSCs are also capable of regulating cells of the immune system by inducing the expression of Fas ligand, which leads to T-cell apoptosis. In a murine colitis model, DPSC treatment reduces colonic inflammation and epithelial ulceration [122].

6. Conclusions

Since the primary role of the dental pulp is thought to be its ability to form hard tissue against external stimuli, it is important to conserve and protect the pulp tissue for long-term maintenance of the tooth. Recent studies have revealed that stem and progenitor cells exist in the dental pulp and can differentiate into hard tissue-forming cells

after stimulation with certain factors. Therefore, a better understanding of the mechanisms underlying odontoblast differentiation from undifferentiated cells may lead to a more effective biologically-activating therapy for pulp cells than afforded by traditional pulp treatments. In addition, the dental pulp has been shown to be a promising cell source for tissue engineering, indicating that further advances in dental pulp biology may contribute to not only better dental therapy but also therapeutic application for regenerative medicine.

Conflict of interest statement

None declared.

Acknowledgement

This work was supported by Grant-in-aid for Scientific Research from Japan Society for the Promotion of Science.

References

[1] Gotjamanos T. Cellular organization in the subodontoblastic zone of the dental pulp. I. A study of cell-free and cell-rich layers in pulps of adult rat and deciduous monkey teeth. Arch Oral Biol 1969;14:1007—10.
[2] Nanci A. Ten Cate's oral histology: development. Struct Funct 2008:192—238.
[3] Lin X, Huang J, Shi Y, Liu W. Tissue engineering and regenerative medicine in applied research: a year in review of 2014. Tissue Eng B Rev 2015;21:177—86.
[4] Kaigler D, Pagni G, Park CH, Braun TM, Holman LA, Yi E, et al. Stem cell therapy for craniofacial bone regeneration: a randomized, controlled feasibility trial. Cell Transplant 2013;22:767—77.
[5] Dhawan J, Rando TA. Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. Trends Cell Biol 2005;15:666—73.
[6] Alvarez-Buylla A, Seri B, Doetsch F. Identification of neural stem cells in the adult vertebrate brain. Brain Res Bull 2002;57:751—8.
[7] Curtis DJ, Salmon JM, Pimanda JE. Concise review: blood relatives: formation and regulation of hematopoietic stem cells by the basic helix-loop-helix transcription factors stem cell leukemia and lymphoblastic leukemia-derived sequence 1. Stem Cells 2012;30:1053—8.
[8] Braun KM, Prowse DM. Distinct epidermal stem cell compartments are maintained by independent niche microenvironments. Stem Cell Rev 2006;2:221—31.
[9] van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol 2009;71:241—60.
[10] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 2000;97:13625—30.
[11] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807—12.
[12] Atari M, Gil-Recio C, Fabregat M, Garcia-Fernandez D, Barajas M, Carrasco MA, et al. Dental pulp of the third molar: a new source of pluripotent-like stem cells. J Cell Sci 2012;125:3343—56.
[13] Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531—5.
[14] Hosoya A, Yoshiba K, Yoshiba N, Hoshi K, Iwaku M, Ozawa H. An immunohistochemical study on hard tissue formation in a subcutaneously transplanted rat molar. Histochem Cell Biol 2003;119:27—35.
[15] Ogawa R, Saito C, Jung HS, Ohshima H. Capacity of dental pulp differentiation after tooth transplantation. Cell Tissue Res 2006;326:715—24.
[16] Hosoya A, Yukita A, Yoshiba K, Yoshiba N, Takahashi M, Nakamura H. Two distinct processes of bone-like tissue formation by dental pulp cells after tooth transplantation. J Histochem Cytochem 2012;60:861—73.
[17] Hosoya A, Nakamura H, Ninomiya T, Hoshi K, Yoshiba K, Yoshiba N, et al. Hard tissue formation in subcutaneously transplanted rat dental pulp. J Dent Res 2007;86:469—74.
[18] Yoshiba K, Yoshiba N, Iwaku M. Class II antigen-presenting dendritic cell and nerve fiber responses to cavities, caries, or caries treatment in human teeth. J Dent Res 2003;82:422—7.
[19] Yoshiba N, Yoshiba K, Nakamura H, Iwaku M, Ozawa H. Immunohistochemical localization of HLA-DR-positive cells in unerupted and erupted normal and carious human teeth. J Dent Res 1996;75:1585—9.
[20] Harada M, Kenmotsu S, Nakasone N, Nakakura-Ohshima K, Ohshima H. Cell dynamics in the pulpal healing process following cavity preparation in rat molars. Histochem Cell Biol 2008;130:773—83.
[21] Hosoya A, Yukita A, Ninomiya T, Hiraga T, Yoshiba K, Yoshiba N, et al. Localization of SUMOylation factors and Osterix in odontoblast lineage cells during dentin formation and regeneration. Histochem Cell Biol 2013;140:201—11.
[22] Kawagishi E, Nakakura-Ohshima K, Nomura S, Ohshima H. Pulpal responses to cavity preparation in aged rat molars. Cell Tissue Res 2006;326:111—22.
[23] Ohshima H, Nakakura-Ohshima K, Takeuchi K, Hoshino M, Takano Y, Maeda T. Pulpal regeneration after cavity preparation, with special reference to close spatio-relationships between odontoblasts and immunocompetent cells. Microsc Res Tech 2003;60:483—90.
[24] Suzuki T, Nomura S, Maeda T, Ohshima H. An immunocytochemical study of pulpal responses to cavity preparation by laser ablation in rat molars by using antibodies to heat shock protein (Hsp) 25 and class II MHC antigen. Cell Tissue Res 2004;315:311—9.
[25] Saito K, Nakatomi M, Ohshima H. Dynamics of bromodeoxyuridine label-retaining dental pulp cells during pulpal healing after cavity preparation in mice. J Endod 2013;39:1250—5.
[26] Kuratate M, Yoshiba K, Shigetani Y, Yoshiba N, Ohshima H, Okiji T. Immunohistochemical analysis of nestin, osteopontin, and proliferating cells in the reparative process of exposed dental pulp capped with mineral trioxide aggregate. J Endod 2008;34:970—4.
[27] Shigetani Y, Yoshiba K, Kuratate M, Takei E, Yoshiba N, Yamanaka Y, et al. Temporospatial localization of dentine matrix protein 1 following direct pulp capping with calcium hydroxide in rat molars. Int Endod J 2014, http://dx.doi.org/10.1111/iej.12351 [Epub ahead of print].
[28] Yoshiba K, Yoshiba N, Iwaku M. Effects of antibacterial capping agents on dental pulps of monkeys mechanically exposed to oral microflora. J Endod 1995;21:16—20.
[29] Yoshiba K, Yoshiba N, Nakamura H, Iwaku M, Ozawa H. Immunolocalization of fibronectin during reparative dentinogenesis in human teeth after pulp capping with calcium hydroxide. J Dent Res 1996;75:1590—7.
[30] Yoshiba N, Yoshiba K, Ohkura N, Hosoya A, Shigetani Y, Yamanaka Y, et al. Expressional alterations of fibrillin-1

during wound healing of human dental pulp. J Endod 2012;38:177—84.
[31] Quispe-Salcedo A, Ida-Yonemochi H, Ohshima H. Effects of a triple antibiotic solution on pulpal dynamics after intentionally delayed tooth replantation in mice. J Endod 2014;40:1566—72.
[32] Shimizu A, Nakakura-Ohshima K, Noda T, Maeda T, Ohshima H. Responses of immunocompetent cells in the dental pulp to replantation during the regeneration process in rat molars. Cell Tissue Res 2000;302:221—33.
[33] Unno H, Suzuki H, Nakakura-Ohshima K, Jung HS, Ohshima H. Pulpal regeneration following allogenic tooth transplantation into mouse maxilla. Anat Rec (Hoboken) 2009;292:570—9.
[34] Robertson A, Lundgren T, Andreasen JO, Dietz W, Hoyer I, Noren JG. Pulp calcifications in traumatized primary incisors. A morphological and inductive analysis study. Eur J Oral Sci 1997;105:196—206.
[35] Shigetani Y, Suzuki H, Ohshima H, Yoshiba K, Yoshiba N, Okiji T. Odontoblast response to cavity preparation with Er:YAG laser in rat molars: an immunohistochemical study. Odontology 2013;101:186—92.
[36] Tanabe K, Yoshiba K, Yoshiba N, Iwaku M, Ozawa H. Immunohistochemical study on pulpal response in rat molars after cavity preparation by Er:YAG laser. Eur J Oral Sci 2002;110:237—45.
[37] Tate Y, Yoshiba K, Yoshiba N, Iwaku M, Okiji T, Ohshima H. Odontoblast responses to GaAlAs laser irradiation in rat molars: an experimental study using heat-shock protein-25 immunohistochemistry. Eur J Oral Sci 2006;114:50—7.
[38] Okiji T, Yoshiba K. Reparative dentinogenesis induced by mineral trioxide aggregate: a review from the biological and physicochemical points of view. Int J Dent 2009;2009:464280.
[39] Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors 2. Factors related to pulpal healing. Endod Dent Traumatol 1995;11:59—68.
[40] Takamori Y, Suzuki H, Nakakura-Ohshima K, Cai J, Cho SW, Jung HS, et al. Capacity of dental pulp differentiation in mouse molars as demonstrated by allogenic tooth transplantation. J Histochem Cytochem 2008;56:1075—86.
[41] Zhao C, Hosoya A, Kurita H, Hu T, Hiraga T, Ninomiya T, et al. Immunohistochemical study of hard tissue formation in the rat pulp cavity after tooth replantation. Arch Oral Biol 2007;52:945—53.
[42] Iohara K, Zheng L, Wake H, Ito M, Nabekura J, Wakita H, et al. A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem Cells 2008;26:2408—18.
[43] Karaoz E, Demircan PC, Saglam O, Aksoy A, Kaymaz F, Duruksu G. Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. Histochem Cell Biol 2011;136:455—73.
[44] Hosoya A, Hiraga T, Ninomiya T, Yukita A, Yoshiba K, Yoshiba N, et al. Thy-1-positive cells in the subodontoblastic layer possess high potential to differentiate into hard tissue-forming cells. Histochem Cell Biol 2012;137:733—42.
[45] Giannopoulou C, Cimasoni G. Functional characteristics of gingival and periodontal ligament fibroblasts. J Dent Res 1996;75:895—902.
[46] Mukai M, Yoshimine Y, Akamine A, Maeda K. Bone-like nodules formed in vitro by rat periodontal ligament cells. Cell Tissue Res 1993;271:453—60.
[47] Nohutcu RM, McCauley LK, Koh AJ, Somerman MJ. Expression of extracellular matrix proteins in human periodontal ligament cells during mineralization in vitro. J Periodontol 1997;68:320—7.
[48] Hosoya A, Ninomiya T, Hiraga T, Yoshiba K, Yoshiba N, Kasahara E, et al. Potential of periodontal ligament cells to regenerate alveolar bone. J Oral Biosci 2010;52:72—80.
[49] Hosoya A, Ninomiya T, Hiraga T, Zhao C, Yoshiba K, Yoshiba N, et al. Alveolar bone regeneration of subcutaneously transplanted rat molar. Bone 2008;42:350—7.
[50] Hamamoto Y, Kawasaki N, Jarnbring F, Hammarstrom L. Effects and distribution of the enamel matrix derivative Emdogain in the periodontal tissues of rat molars transplanted to the abdominal wall. Dent Traumatol 2002;18:12—23.
[51] Kawasaki N, Hamamoto Y, Nakajima T, Irie K, Ozawa H. Periodontal regeneration of transplanted rat molars after cryopreservation. Arch Oral Biol 2004;49:59—69.
[52] Bronckers AL, D'Souza RN, Butler WT, Lyaruu DM, van Dijk S, Gay S, et al. Dentin sialoprotein: biosynthesis and developmental appearance in rat tooth germs in comparison with amelogenins, osteocalcin and collagen type-I. Cell Tissue Res 1993;272:237—47.
[53] D'Souza RN, Bronckers AL, Happonen RP, Doga DA, Farach-Carson MC, Butler WT. Developmental expression of a 53 KD dentin sialoprotein in rat tooth organs. J Histochem Cytochem 1992;40:359—66.
[54] Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149—55.
[55] Saito K, Nakatomi M, Ida-Yonemochi H, Kenmotsu S, Ohshima H. The expression of GM-CSF and osteopontin in immunocompetent cells precedes the odontoblast differentiation following allogenic tooth transplantation in mice. J Histochem Cytochem 2011;59:518—29.
[56] Bessho K, Tanaka N, Matsumoto J, Tagawa T, Murata M. Human dentin-matrix-derived bone morphogenetic protein. J Dent Res 1991;70:171—5.
[57] Butler WT, Mikulski A, Urist MR, Bridges G, Uyeno S. Non-collagenous proteins of a rat dentin matrix possessing bone morphogenetic activity. J Dent Res 1977;56:228—32.
[58] Hu CC, Zhang C, Qian Q, Tatum NB. Reparative dentin formation in rat molars after direct pulp capping with growth factors. J Endod 1998;24:744—51.
[59] Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med 2001;12:425—37.
[60] Sloan AJ, Rutherford RB, Smith AJ. Stimulation of the rat dentine—pulp complex by bone morphogenetic protein-7 in vitro. Arch Oral Biol 2000;45:173—7.
[61] Inoue T, Shimono M. Repair dentinogenesis following transplantation into normal and germ-free animals. Proc Finn Dent Soc 1992;88(Suppl. 1):183—94.
[62] Yamamura T. Differentiation of pulpal cells and inductive influences of various matrices with reference to pulpal wound healing. J Dent Res 1985;64. Spec No: 530-40.
[63] Yamazoe T, Aoki K, Simokawa H, Ohya K, Takagi Y. Gene expression of bone matrix proteins in a calcified tissue appeared in subcutaneously transplanted rat dental pulp. J Med Dent Sci 2002;49:57—66.
[64] Bridges JB, Pritchard JJ. Bone and cartilage induction in the rabbit. J Anat 1958;92:28—38.
[65] Ishikawa Y, Ida-Yonemochi H, Suzuki H, Nakakura-Ohshima K, Jung HS, Honda MJ, et al. Mapping of BrdU label-retaining dental pulp cells in growing teeth and their regenerative capacity after injuries. Histochem Cell Biol 2010;134:227—41.
[66] Kenmotsu M, Matsuzaka K, Kokubu E, Azuma T, Inoue T. Analysis of side population cells derived from dental pulp tissue. Int Endod J 2010;43:1132—42.
[67] Balic A, Aguila HL, Caimano MJ, Francone VP, Mina M. Characterization of stem and progenitor cells in the dental

pulp of erupted and unerupted murine molars. Bone 2010;46:1639—51.
[68] Kaneko R, Akita H, Shimauchi H, Sasano Y. Immunohistochemical localization of the STRO-1 antigen in developing rat teeth by light microscopy and electron microscopy. J Electron Microsc (Tokyo) 2009;58:363—73.
[69] Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003;18:696—704.
[70] Yalvac ME, Ramazanoglu M, Rizvanov AA, Sahin F, Bayrak OF, Salli U, et al. Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-, adipo- and neurogenesis. Pharmacogenomics J 2010;10:105—13.
[71] Bottcher DE, Scarparo RK, Batista Jr EL, Fossati AC, Grecca FS. Histologic evaluation and immunohistochemical localization of STRO-1 and BMP-4 in rat immature teeth: a comparison between vital and induced pulp necrosis. Arch Oral Biol 2013;58:1174—9.
[72] Li L, Kwon HJ, Harada H, Ohshima H, Cho SW, Jung HS. Expression patterns of ABCG2, Bmi-1, Oct-3/4, and Yap in the developing mouse incisor. Gene Expr Patterns 2011;11:163—70.
[73] Yoshiba N, Yoshiba K, Ohkura N, Shigetani Y, Takei E, Hosoya A, et al. Immunohistochemical analysis of two stem cell markers of alpha-smooth muscle actin and STRO-1 during wound healing of human dental pulp. Histochem Cell Biol 2012;138:583—92.
[74] Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng 2006;12:2813—23.
[75] Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8:191—9.
[76] Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 2003;82:976—81.
[77] Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008;34:962—9.
[78] Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430—6.
[79] Horibe H, Murakami M, Iohara K, Hayashi Y, Takeuchi N, Takei Y, et al. Isolation of a stable subpopulation of mobilized dental pulp stem cells (MDPSCs) with high proliferation, migration, and regeneration potential is independent of age. PLOS ONE 2014;9:e98553.
[80] Iohara K, Murakami M, Takeuchi N, Osako Y, Ito M, Ishizaka R, et al. A novel combinatorial therapy with pulp stem cells and granulocyte colony-stimulating factor for total pulp regeneration. Stem Cells Transl Med 2013;2:521—33.
[81] Murakami M, Horibe H, Iohara K, Hayashi Y, Osako Y, Takei Y, et al. The use of granulocyte-colony stimulating factor induced mobilization for isolation of dental pulp stem cells with high regenerative potential. Biomaterials 2013;34:9036—47.
[82] Nakashima M, Iohara K. Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. J Endod 2014;40:S26—32.
[83] Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 1996;183:1797—806.
[84] Honda MJ, Nakashima F, Satomura K, Shinohara Y, Tsuchiya S, Watanabe N, et al. Side population cells expressing ABCG2 in human adult dental pulp tissue. Int Endod J 2007;40:949—58.
[85] Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M. Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. Stem Cells 2006;24:2493—503.
[86] Wang J, Wei X, Ling J, Huang Y, Huo Y, Zhou Y. The presence of a side population and its marker ABCG2 in human deciduous dental pulp cells. Biochem Biophys Res Commun 2010;400:334—9.
[87] Ninomiya T, Hiraga T, Hosoya A, Ohnuma K, Ito Y, Takahashi M, et al. Enhanced bone-forming activity of side population cells in the periodontal ligament. Cell Transplant 2014;23:691—701.
[88] Kawanabe N, Murakami K, Takano-Yamamoto T. The presence of ABCG2-dependent side population cells in human periodontal ligaments. Biochem Biophys Res Commun 2006;344:1278—83.
[89] Hristov M, Erl W, Weber PC. Endothelial progenitor cells: isolation and characterization. Trends Cardiovasc Med 2003;13:201—6.
[90] Iohara K, Zheng L, Ito M, Ishizaka R, Nakamura H, Into T, et al. Regeneration of dental pulp after pulpotomy by transplantation of CD31(−)/CD146(−) side population cells from a canine tooth. Regen Med 2009;4:377—85.
[91] Ishizaka R, Hayashi Y, Iohara K, Sugiyama M, Murakami M, Yamamoto T, et al. Stimulation of angiogenesis, neurogenesis and regeneration by side population cells from dental pulp. Biomaterials 2013;34:1888—97.
[92] Ishizaka R, Iohara K, Murakami M, Fukuta O, Nakashima M. Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials 2012;33:2109—18.
[93] Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, et al. Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue Eng A 2011;17:1911—20.
[94] Nakashima M, Iohara K. Regeneration of dental pulp by stem cells. Adv Dent Res 2011;23:313—9.
[95] Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 2000;102:451—61.
[96] Mutoh N, Nakatomi M, Ida-Yonemochi H, Nakagawa E, Tani-Ishii N, Ohshima H. Responses of BrdU label-retaining dental pulp cells to allogenic tooth transplantation into mouse maxilla. Histochem Cell Biol 2011;136:649—61.
[97] Saito K, Nakatomi M, Kenmotsu S, Ohshima H. Allogenic tooth transplantation inhibits the maintenance of dental pulp stem/progenitor cells in mice. Cell Tissue Res 2014;356:357—67.
[98] Saito K, Ishikawa Y, Nakakura-Ohshima K, Ida-Yonemochi H, Nakatomi M, Kenmotsu S, et al. Differentiation capacity of BrdU label-retaining dental pulp cells during pulpal healing following allogenic transplantation in mice. Biomed Res 2011;32:247—57.
[99] Ishikawa Y, Ida-Yonemochi H, Nakakura-Ohshima K, Ohshima H. The relationship between cell proliferation and differentiation and mapping of putative dental pulp stem/progenitor cells during mouse molar development by chasing BrdU-labeling. Cell Tissue Res 2012;348:95—107.
[100] Hoshi K, Amizuka N, Oda K, Ikehara Y, Ozawa H. Immunolocalization of tissue non-specific alkaline phosphatase in mice. Histochem Cell Biol 1997;107:183—91.

[101] Yoshiki S, Kurahashi Y. A light and electron microscopic study of alkaline phosphatase activity in the early stage of dentinogenesis in the young rat. Arch Oral Biol 1971;16: 1143—54.
[102] Avery JK. Oral development and histology. 3rd ed. New York: Thieme Publishing Group; 2001.
[103] Arai F, Ohneda O, Miyamoto T, Zhang XQ, Suda T. Mesenchymal stem cells in perichondrium express activated leukocyte cell adhesion molecule and participate in bone marrow formation. J Exp Med 2002;195:1549—63.
[104] Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, et al. Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell 2008;13:153—66.
[105] Nakamura H, Yukita A, Ninomiya T, Hosoya A, Hiraga T, Ozawa H. Localization of Thy-1-positive cells in the perichondrium during endochondral ossification. J Histochem Cytochem 2010;58:455—62.
[106] Stevenson KS, McGlynn L, Hodge M, McLinden H, George WD, Davies RW, et al. Isolation, characterization, and differentiation of thy1.1-sorted pancreatic adult progenitor cell populations. Stem Cells Dev 2009;18:1389—98.
[107] Balic A, Aguila HL, Mina M. Identification of cells at early and late stages of polarization during odontoblast differentiation using pOBCol3.6GFP and pOBCol2.3GFP transgenic mice. Bone 2010;47:948—58.
[108] Choi JK, Hwang HI, Jang YJ. The efficiency of the in vitro osteo/dentinogenic differentiation of human dental pulp cells, periodontal ligament cells and gingival fibroblasts. Int J Mol Med 2015;35:161—8.
[109] Tamaki Y, Nakahara T, Ishikawa H, Sato S. In vitro analysis of mesenchymal stem cells derived from human teeth and bone marrow. Odontology 2013;101:121—32.
[110] Teti G, Salvatore V, Ruggeri A, Manzoli L, Gesi M, Orsini G, et al. In vitro reparative dentin: a biochemical and morphological study. Eur J Histochem 2013;57:e23.
[111] Ohshima H, Nakakura-Ohshima K, Yamamoto H, Maeda T. Alteration in the expression of heat shock protein (Hsp) 25-immunoreactivity in the dental pulp of rat molars following tooth replantation. Arch Histol Cytol 2001;64:425—37.
[112] Heyeraas KJ, Kvinnsland I, Byers MR, Jacobsen EB. Nerve fibers immunoreactive to protein gene product 9.5, calcitonin gene-related peptide, substance P, and neuropeptide Y in the dental pulp, periodontal ligament, and gingiva in cats. Acta Odontol Scand 1993;51:207—21.
[113] Fried K, Nosrat C, Lillesaar C, Hildebrand C. Molecular signaling and pulpal nerve development. Crit Rev Oral Biol Med 2000;11:318—32.
[114] Nosrat CA, Fried K, Ebendal T, Olson L. NGF, BDNF, NT3, NT4 and GDNF in tooth development. Eur J Oral Sci 1998; 106(Suppl. 1):94—9.
[115] Arany S, Koyota S, Sugiyama T. Nerve growth factor promotes differentiation of odontoblast-like cells. J Cell Biochem 2009;106:539—45.
[116] Mitsiadis TA, Luukko K. Neurotrophins in odontogenesis. Int J Dev Biol 1995;39:195—202.
[117] Woodnutt DA, Wager-Miller J, O'Neill PC, Bothwell M, Byers MR. Neurotrophin receptors and nerve growth factor are differentially expressed in adjacent nonneuronal cells of normal and injured tooth pulp. Cell Tissue Res 2000;299:225—36.
[118] Ikeda E, Yagi K, Kojima M, Yagyuu T, Ohshima A, Sobajima S, et al. Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. Differentiation 2008;76:495—505.
[119] Bardin N, George F, Mutin M, Brisson C, Horschowski N, Frances V, et al. S-Endo 1, a pan-endothelial monoclonal antibody recognizing a novel human endothelial antigen. Tissue Antigens 1996;48:531—9.
[120] Sugiyama M, Iohara K, Wakita H, Hattori H, Ueda M, Matsushita K, et al. Dental pulp-derived CD31(−)/CD146(−) side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Eng A 2011;17:1303—11.
[121] Yalvac ME, Yarat A, Mercan D, Rizvanov AA, Palotas A, Sahin F. Characterization of the secretome of human tooth germ stem cells (hTGSCs) reveals neuro-protection by fine-tuning micro-environment. Brain Behav Immun 2013;32:122—30.
[122] Zhao Y, Wang L, Jin Y, Shi S. Fas ligand regulates the immunomodulatory properties of dental pulp stem cells. J Dent Res 2012;91:948—54.