Review Article

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

Akihiro Hosoya (DDS, PhD)*, Hiroaki Nakamura (DDS, PhD)

Department of Oral Histology, Matsumoto Dental University, 1780 Gobara Hirooka, Shiojiri, Nagano 399-0781, Japan

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.

© 2015 Japanese Association for Dental Science. Published by Elsevier Ltd. All rights reserved.

Contents

1. Introduction ................................................................................................................................................. 76
2. Pulp calcification .............................................................................................................................................. 76
3. Stem and progenitor cells in the dental pulp .............................................................................................. 77
   3.1. Dental pulp stem cells (DPSCs) .................................................................................................................. 78
   3.2. DPSCs mobilized by G-CSF (MDPCs) ........................................................................................................ 78
   3.3. Side population (SP) cells .......................................................................................................................... 78
   3.4. Label-retaining cells (LRCs) ...................................................................................................................... 78
   3.5. Cells in the subodontoblastic layer .......................................................................................................... 78

* Corresponding author. Tel.: +81 263512043; fax: +81 263533456.
E-mail address: hosoya@po.mdu.ac.jp (A. Hosoya).

http://dx.doi.org/10.1016/j.jdsr.2015.03.002
1882-7616/© 2015 Japanese Association for Dental Science. Published by Elsevier Ltd. All rights reserved.
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 Wiel) 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 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,
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 cell 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 STR0-1, ABCG2, CD90, α-smooth muscle actin, and Bmi1 [12,67–70]. Among them, STR0-1 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

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental animal models of pulp calcification.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant</td>
<td>Recipient</td>
</tr>
<tr>
<td>Whole tooth</td>
<td>Hypodermis</td>
</tr>
<tr>
<td>Whole tooth</td>
<td>Tooth socket</td>
</tr>
<tr>
<td>Tooth crown</td>
<td>Submucosa</td>
</tr>
<tr>
<td>Pulp</td>
<td>Hypodermis</td>
</tr>
</tbody>
</table>
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 differentiate into odontoblast-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 odontoblastic 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
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 re plantation [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


