Differentiation of Dental Pulp Stem Cells into Regular-Shaped Dentin-Pulp Complex Induced by Tooth Germ Cell Conditioned Medium

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ABSTRACT

Investigations of the odontoblast phenotype are hindered by obstacles such as the limited number of odontoblasts within the dental pulp and the difficulty in purification of these cells. Therefore, it is necessary to develop a cell culture system in which the local environment is inductive and can promote dental pulp stem cells (DPSCs) to differentiate into odontoblast lineage. In this study, we investigated the effect of conditioned medium from developing tooth germ cells (TGCs) on the differentiation and dentinogenesis of DPSCs both in vitro and in vivo. DPSCs were enzymatically isolated from the lower incisors of 4-week-old Sprague-Dawley rats and co-cultured with TGC conditioned medium (TGC-CM). The cell phenotype of induced DPSCs presents many features of odontoblasts, as assessed by the morphologic appearance, cell cycle modification, increased alkaline phosphatase level, synthesis of dentin sialoprotein, type I collagen and several other noncollagenous proteins, expression of the dentin sialophosphoprotein and dentin matrix protein 1 genes, and the formation of mineralized nodules in vitro. The induced DPSC pellets in vivo generated a regular-shaped dentin-pulp complex containing distinct dentinal tubules and predentin, while untreated pellets spontaneously differentiated into bone-like tissues. To our knowledge, this is the first study to mimic the dentinogenic microenvironment from TGCs in vitro, and our data suggest that TGC-CM creates the most odontogenic microenvironment, a feature essential and effective for the regular dentinogenesis mediated by DPSCs.

INTRODUCTION

Dentinogenesis is modulated by highly differentiated postmitotic odontoblasts originating from neural crest–derived mesenchymal cells during tooth development. The commitment and differentiation of odontoblasts are regulated by sequential interactions between mesenchymal cells and epithelial cells in the oral environment, while the terminal differentiation of these cells is characterized by several steps, including the withdrawal from the cell cycle, elongation and cytologic polarization, and transcriptional and translational modifications that allow these cells to differentiate into odontoblasts.

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synthesize the predentin matrix.1–7 Both growth factors and extracellular matrix components are thought to play an important role in this cellular commitment pathway.3,5,8,9 These growth factors, which include transforming growth factors (TGFs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), and platelet-derived growth factor, induce odontoblast differentiation and reparative dentin formation in cultured pulp cells and experimental animals.3,5,8,10 Dental pulp stem cells (DPSCs) have been isolated as multipotent stem cells, which have the potential for self-renewal and multilineage differentiation into such cells as adipocytes, neural-like cells, and odontoblast-like cells.11–15 The fate of DPSCs appears to be determined in part by intrinsic genetic programs and also by the influence of growth factors located in the microenvironment.9,13,16 Previous studies have proved that many single factors can influence the differentiation of DPSCs in vitro.8,17,18 But it is still unclear whether a combination of these multiple factors can affect the dentinogenesis of DPSCs in vivo. For this purpose, this study was designed to mimic the dentinogenic microenvironment and test the hypothesis that soluble factors released from dental epithelial and mesenchymal cells in developing tooth germ cells (TGCs) may play a paramount role in the differentiation and dentinogenesis process mediated by DPSCs. The characteristics of DPSCs were assessed by in vitro morphologic appearance, gene and protein expression of odontoblast phenotype, alkaline phosphatase (ALP) activity, formation of mineralized nodules, analysis of cell cycle, and the evaluation of in vivo dentinogenesis.

MATERIALS AND METHODS

TGC isolation and preparation of TGC conditioned medium

All procedures were carried out according to the guidelines of the Animal Care Committee of Fourth Military Medical University. The TGCs were isolated as described elsewhere,19 with some modifications. Twenty lower incisor TGCs were carefully isolated from 2-day postnatal Sprague-Dawley rat pups by using dental explorer and were minced into less than 1-mm pieces in 0.01 M phosphate-buffered saline (PBS; GIBCO-BRL, Bethesda, MD). Tooth bud tissues were digested with type I collagenase (0.62 mg/mL; Sigma, St. Louis, MO) for 1 h. Cells were dissociated by gentle trituration, collected by centrifugation, and washed twice in Dulbecco’s modified Eagle medium (DMEM; GIBCO-BRL) containing 10% fetal bovine serum (FBS), 0.292 mg/mL Glutamine (Invitrogen, Carlsbad, CA), 100 units/mL penicillin G, 100 μg/mL streptomycin, 2.5 μg/mL ascorbic acid. Single-cell suspensions were generated by filtration through a 70 μm strainer, washed again with DMEM supplemented with 10% FBS, then placed into 75 cm² culture flasks (Costar, Cambridge, MA) at 1 × 10⁵ cells/mL and grown in 5% carbon dioxide at 37°C. The culture medium of primary TGCs containing both epithelial and mesenchymal cells at 70% confluence was changed every 24 h until full confluence for collecting the supernatant, which would be filtered through a 0.22 μm Millipore strainer (Carrigtwohill Co., Cork, Ireland), followed by spinning at 2000 g for 20 min. The supernatant was mixed with equal volume of fresh DMEM supplemented with 10% FBS and used as TGC conditioned medium (TGC-CM) for DPSC culture. The DMEM supplemented with 10% FBS was used as a control medium. The experiment for TGC culture was repeated 45 times for collecting the medium, and the average time period for culture was 7 days.

DPSC isolation and establishment of co-culture system

Multicolly derivived DPSCs were isolated and cultured as reported elsewhere.11,12 The dental pulps were extracted from the lower incisors of 4-week-old male Sprague-Dawley rats, and were physically separated from the enamel organs and apical buds,20 which contain dental epithelial tissues, to obtain pure DPSCs. DPSCs in the secondary passage were co-cultured with TGC-CM, which would be regularly changed every 24 h. Cells in the conditioned medium were routinely observed and photographed under phase-contrast inverted microscopy (Olympus Optical Co. Ltd., Tokyo, Japan) to evaluate their appearance. The stem cell nature of isolated cells was confirmed by positive immunostaining for STRO-1 (monoclonal antibody; Sigma; data not shown), the putative stem cell marker, according to the manufacturer’s protocol.

Flow cytometry analysis

After 7-day co-culture with TGC-CM, DPSCs were collected by exposure to trypsin/ ethylenediaminetetraacetic acid (Life Technologies, Inc., Rockland, MD) for 5 min and centrifuged at 600 g for 6 min. Cell precipitates were washed twice with 0.01 M PBS and resuspended in 1 mL physiologic saline by repeated vibration to ensure a single cell suspension. Then, 2 mL of cold dehydrated alcohol was mixed quickly with the cell suspension to fix cells at −4°C for 24–48 h. Finally, the cells were washed twice again with PBS as mentioned previously, stained with propidium iodide (100 μg/mL; Sigma) at 4°C for 30 min, and subjected to Elite ESP flow cytometry (Beckman Coulter Inc., Fullerton, CA). One million cells were counted per sample, and the fraction of cells in the G1, S, and G2 phases of cell cycle was analyzed.

ALP activity assay

The DPSCs were seeded at a density of 1 × 10³ cells/well in 96-well plates. After 1, 3, 5, 7, 10, and 14 days of co-culture, the ALP activity of DPSCs was detected using an ALP assay kit (JianCheng Co., Nanjing, China) according to the manufacturer’s instruction. The results were measured
Western blot for DSP and DMP 1

Total cellular proteins from DPSCs after 7-day induction, untreated DPSCs, and 2-day postnatal rat tooth germ cells (positive control) were prepared respectively with lysis buffer (pH, 8.0) containing 1% NP-40 (Sangon Co., Shanghai, China), 50 mM trishydroxymethylaminomethane (Tris)-hydrogen chloride (HCl), 150 mM sodium chloride (NaCl), 0.1 mM phenylme-thylsulfonyl fluoride, and 1 µg/ml aprotinin (Sigma). The detergent-soluble fractions were subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels according to standard protocol. Separated proteins in gels were stained with Coomassie blue or electrotransferred to nitrocellulose membranes using consistent voltage 25 V for 20 min. After blocking in 5% (w/v) skim milk diluted in TRIS-buffered saline (TBS) buffer (blocking buffer, 50 mM Tris-HCl and 150 mM NaCl) at room temperature for 2 h, the membranes were incubated overnight at 4°C with rabbit anti-rat DSP and goat anti-rat DMP1 at 1:500 dilution. The membranes were washed with TBST (0.2% Tween-20 in blocking buffer) and incubated with horseradish peroxidase conjugated goat anti-rabbit IgG and rabbit anti-goat IgG (Boster Biotechnology Co., Wuhan, China) diluted in blocking buffer at 37°C for 2 h. After washing, the membranes were subjected to enhanced chemoluminescence reagents (Santa Cruz Biotechnology, Inc.) and exposed to Kodak x-ray film.

Reverse transcriptase polymerase chain reaction for dentin sialophosphoprotein and DMP 1

DPSCs at 90% confluence were harvested after 7-day co-culture with TGC-CM. Total RNA isolation, first-strand complementary DNA synthesis, and polymerase chain reaction (PCR) processes were performed as reported elsewhere. Primer sequences for dentin sialophosphoprotein (DSPP) (GenBank accession no. NM_012790), DMP 1 (GenBank accession no. NM_203493), and β-actin (GenBank accession no. NM_031144) were as follows: 1) DSPP-sense, 5'-TAAGGACAAGGACGAATC-3', and DSPP-antisense, 5'-ACTGCTGTACTGCTTTC-3'; 2) DMP 1-sense, 5'-CG GTCTGGTGCTCCTGCTTTCT-3'; and DMP 1-antisense, 5'-GTTCCCTTGGGGCTATCCCTT-3'; 3) β-actin-sense, 5'-GA GCCTTTCAACACCCCCAGC-3', and β-actin-antisense, 5'-CATAGCAAGCATCTCTTACT-3', used as an internal control. The following PCR cycles were used: denaturation, 94°C for 45 s; annealing, 57°C for 45 s; and extension, 72°C for 60 s, for 30 cycles, then 74°C for 5 min. The PCR products were separated by using 2% agarose gel electrophoresis and were stained with ethidium bromide; digital images were taken on ultraviolet background. The expected product sizes from these primers were 472 base pairs (DSPP), 705 base pairs (DMP 1), and 283 base pairs (β-actin), respectively. The PCR products were further confirmed by sequencing (Sangon Biotechnology Co.).

In vivo differentiation of DPSC pellets

DPSCs co-cultured with TGC-CM for 7 days were harvested by 0.25% trypsin (GIBCO-BRL) for 7 min at 37°C and washed twice with TGC-CM. The cell pellet culture was performed as described elsewhere, with some modifications. Briefly, 2-mL conditioned medium containing 1 × 10⁶ cells was centrifuged in a 10-mL conical polypropylene tube (Asahi Techno Glass Corp., Tokyo, Japan) at 800 g for...
5 min. Cell pellets in tubes, approximately 1.5 mm\(^3\) in size, were maintained in TGC-CM at 37\(^\circ\)C for 5 h to make them well congregated and favorable for transplantation procedures; the pellets were then seeded directly into the renal capsule with modified pipette tips on the left side of 8-week-old allogenic Sprague-Dawley rat hosts. As a control, the untreated DPSC pellets were implanted into the other side of the same host. Transplantation procedures were performed as described elsewhere.\textsuperscript{23} We implanted 67 cell pellets into the renal capsules, and the development of these pellets was evaluated after 3 and 14 days of incubation by hematoxylin-eosin (H&E) staining. The control groups, C1 and C2, consisted of 12 cell pellets after 3-day incubation (C1) and 20 pellets after 14-day incubation (C2). The experimental groups, E1 and E2, consisted of 12 cell pellets after 3-day incubation (E1) and 23 pellets after 14-day incubation (E2).

Statistical analysis

Statistical significance was assessed by independent-samples \(t\)-test.

RESULTS

Effect of TGC-CM on morphologic appearance and cell cycle of DPSCs in vitro

To test whether TGC-CM could induce the odontoblast-like differentiation, DPSCs were co-cultured with TGC-CM for 7 days and then subjected to cell cycle analysis. After 4-day co-culture, the initially polymorphic DPSCs (Fig. 1A) displayed a columnar, elongated appearance; long cellular processes; and a tendency to align themselves in straight parallel lines (Fig. 1B). Some induced cells showed long single cellular processes (Fig. 1B). With culture time, the cells were more columnar, with a long spindle shape and tightly packed arrangement at day 7 (Fig. 1D). In general, cells in control medium had no apparent morphologic changes during 7-day routine culture (Fig. 1C).

Representative histograms of flow cytometry at day 7 revealed the responses of induced cells as a typical distribution of 3 phases with double peaks (Fig. 2A), corresponding to cells in G1 and G2 phases, while control DPSCs

![FIG. 1](image1.png)

**FIG. 1.** Effect of tooth germ cell conditioned medium on the morphology of dental pulp stem cells (DPSCs). (A) Untreated DPSCs at day 4 were apparently polymorphic in shape. Most cells were spindle-shaped and fibroblast-like, and some were cuboidal or polygonal in appearance. (B) Induced DPSCs at day 4 became elongated, with long cytoplasmic processes, and tended to align themselves in parallel lines. (C) Untreated DPSCs had no obvious morphologic changes at day 7. (D) At day 7, induced DPSCs were mostly monopolar or bipolar and were arranged in more orderly fashion than control cells. Scale bars = 50 \(\mu\)m. Color images available online at www.liebertpub.com/ten.

![FIG. 2](image2.png)

**FIG. 2.** Effect of tooth germ cell conditioned medium on the cell cycle of dental pulp stem cells (DPSCs). (A) Representative cell cycle distributions of treated DPSCs. G1, S, and G2 represent different phases. (B) Untreated DPSCs displayed only one peak of G1 phase.
displayed only 1 peak of G1 phase (Fig. 2B). A higher percentage of cells in S (35.6%) and G2 (16.8%) phases and a lower percentage in G1 phase (47.6%) were detected in induced cells compared with untreated cells (Fig. 2A and B), suggesting that many induced cells shifted from G1 phase to G2 or S phases, and tended to withdraw from the cell cycle. In control DPSCs, almost all cells stayed in G1 phase. Taken together, TGC-CM could induce DPSCs to make some morphologic changes similar to odontoblasts and some modifications in the cell cycle of DPSCs at the same time.

**Effect of TGC-CM on ALP activity and mineralization behavior of DPSCs in vitro**

It is well known that ALP is a marker for odontoblast differentiation because odontoblasts show much higher ALP activity than dental undifferentiated mesenchymal cells. Our data clearly demonstrated significantly ($p < .01$) more ALP activity in treated group than in the control group at almost every time point except for day 1 (Fig. 3), indicating that the induced cells may have started the initial differentiation at day 3. The ALP activity in induced DPSCs quickly increased as the cells differentiated, reached peak level at day 7, then declined gradually when mineralized nodules from differentiated DPSCs came into being at day 14 (Figs. 3 and 4B). The ALP activity in the control group steadily increased with culture time as the cells grew more confluent (Fig. 3).

Mineralization of the dentin matrix is a hallmark of functional and fully differentiated odontoblasts. However, DPSCs could not spontaneously form the mineralized nodules during 14-day routine culture (Fig. 4A). With the TGC-CM treatment, DPSCs were induced to mineralize at day 14, as indicated by von Kossa stain (Fig. 4B). Our findings indicate that TGC-CM can upregulate ALP activity and promote the secretion of extracellular matrix of DPSCs to form the mineralized nodules.

**Effect of TGC-CM on protein and gene expression of DPSCs in vitro**

We used several methods, such as immunocytochemical staining, Western blot, and reverse transcriptase PCR to detect the expression of proteins and genes responsible for odontoblast differentiation. Our results showed that treated cells after 7-day induction stained positively for cytoplasmic DSP, DMP 1, BSP, ON, OPN, and Col I (Fig. 5A–F). A-F) No obvious staining could be observed in control cells. Scale bars = 50 μm. Color images available online at www.liebertpub.com/ten.
respectively), whereas no staining was detected in untreated cells (Fig. 5 A’–F’, respectively) by immunocytochemical technique. Some morphologically different cells in the induced group expressing the same odontoblast-related proteins indicate that these induced cells may belong to the different developmental stages of odontoblast lineage (Fig. 5). The results of sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blot further confirmed the existence of DSP and DMP 1 in experimental cells after 7-day coculture (Fig. 6A and B), suggesting that induced DSPCs with odontoblast-like appearance were able to synthesize and secrete these major dentin proteins. No traces of DSP and DMP 1 expression were noted in the control group (Fig. 6B).

To confirm the odontoblast phenotype of induced odontoblast-like cells, reverse transcriptase PCR was performed to investigate the expression of DSPP and DMP 1 genes. Gene expression for DSPP and DMP 1 could be detected in the induced cells at day 7 but not the control cells (Fig. 6C). These findings indicate that TGC-CM can induce the differentiation of DSPCs by stimulating the synthesis of DSP, Col I and other non-collagenous proteins, as well as by promoting the expression of DSPP and DMP 1 transcripts. Collectively, these induced DSPCs present several crucial features of odontoblasts in vitro.

**Effect of TGC-CM on differentiation of DSPCs in vivo**

On the basis of findings from the in vitro experiments, an in vivo evaluation of induced DPSCs was performed to verify whether the induced DSPCs still maintain their differentiated state and form the dental tissues. The cell pellets in renal capsules proliferated well, and morphologic examination presented little evidence of inflammation or rejection. Untreated DSPCs had no orderly arrangement (Fig. 7A) at day 3 and generally developed osteotypic tissues at day 14; in these tissues, osteocyte-like lacunae were clear and numerous osteoblast-like cells lined up along the edge (Fig. 7B and C). Induced DPSCs appeared to maintain their differentiated state and continued to function in vivo 2 weeks. These cells were well organized in a palisade-like parallel arrangement (Fig. 7D) at day 3, and formed a dentin-pulp complex with a regular outline (Fig. 7E) containing distinctive dentinal tubules, predentin, and pulp cavity (Fig. 7F) at day 14. Odontoblast-like cells presented only on one side of the predentin-dentin (Fig. 7E). The percentages of successful experiments in the induced group are 50% (6 of 12) at day 3 and 35% (8 of 23) at day 14, while no dental structures were formed in control group and all untreated pellets (20 of 20) at day 14 developed into bone-like tissues. Our in vivo data showed that induced DSPC pellets reliably differentiated into a highly ordered dentin-pulp complex while untreated pellets had a tendency to self-differentiate into osteotypic tissues.

**DISCUSSION**

Rodent incisors are continuously growing teeth that are maintained by the cell proliferation both at the apical end and the attrition of the incisal edge. This type of tooth has a special epithelial structure for the maintenance of stem cells, showing the bulbous epithelial protrusion termed “apical bud” at the apical end. Previous studies have shown that the molecular signals regulating the fate of adult stem cells (e.g., Notch-1, TGF-βs, bone morphogenetic proteins [BMPs], and FGFs) are expressed permanently in the epithelial structure and the surrounding mesenchyme of apical bud, whose function is similar to that of the prenatal tooth bud. Thus, by using the TGCs from the developing rat incisors, it seems likely that the differentiation of DSPCs into cells with some salient features of native odontoblasts can be induced.

![FIG. 6. Results of sodium dodecyl sulfate polyacrylamide gel electrophoresis, Western blot, and reverse transcriptase polymerase chain reaction (RT-PCR). (A) Sodium dodecyl sulfate polyacrylamide gel electrophoresis gels were stained by Coomassie blue reagent: lane 1, marker; lane 2, untreated cells; lane 3, 2dpn rat tooth germs (positive control); lane 4, treated cells. (B) Western blot was performed from protein extracts with anti–dentin sialoprotein (DSP) and anti-dentin matrix protein 1 (DMP 1) antibodies: lane 1, untreated dental pulp stem cells (DSPCs); lane 2, 2dpn rat tooth germs (positive control); lane 3, treated DSPCs. (C) Electrophoresis of RT-PCR products: lane 1 and 2, DMP 1 and DSPP in treated DSPCs; lane 3 and 4, DMP 1 and DSPP in untreated DSPSCs. β-actin was used as an internal control for each group. bp: base pair. Color images available online at www.liebertpub.com/ten.](image)
Dental pulp stem cells differentiated into dentin-pulp complex

Figure 7. Hematoxylin-eosin–stained control and experimental implants. (A) Histologic analysis of the 3-day implants exhibits no regular arrangement of control cells. (B and C) Almost all control pellets generated osteotypic tissues with distinct osteocyte-like lacunae (black arrows) and osteoblast-like cells (open arrows) at day 14. (D) 3-day implants of induced pellets showed odontoblasts (OB) in a palisade-like parallel arrangement. (E) 14-day pellet samples of induced cells developed a distinctive dentin-pulp complex structure with regular outline and columnar odontoblasts (OB) arrayed in an orderly manner alongside the predentin. (F) The dentinal tubules (black arrows), predentin (PD), dentin (D), and blood vessels (BV) are apparent in the dentin-pulp complex. Scale bars = 50 μm. Color images available online at www.liebertpub.com/ten.

Odontoblasts derive from dental papilla cells, exhibit a tall columnar shape, and establish a continuous single layer with a clear epithelioid appearance. Our observations have clearly confirmed that DPSCs can differentiate into odontoblast-like cells following the induction of TGC-CM, suggesting that induced DPSCs are similar to the embryonic dental papilla cells residing within the dental pulp. Induced DPSCs provide a unique opportunity to determine the mechanisms responsible for the odontoblast differentiation and will be useful for the tissue engineering of dentin regeneration or even for the further identification of odontoblast-specific genes. The cell cycle results showed that many of these odontoblast-like DPSCs tended to exit from the cell cycle. This phenomenon is similar to the gradient of odontoblast differentiation observed during dentinogenesis, in which competent preodontoblasts emerge progressively by sequential withdrawal from the cell cycle. DPSCs are multipotential stem cells whose differentiation is modulated by the local environment that they inhabit. The TGC-CM in this study successfully provided a growth condition with multiple molecular signals or growth factors necessary for DPSC differentiation and proliferation. The cell phenotype of induced DPSCs presents several crucial characteristics of odontoblasts, as provided by the morphologic changes; cell cycle modification; increased ALP activity; synthesis of DSP, Col I, and other noncollagenous proteins; expression of the DSPP and DMP 1 transcripts; and formation of mineralized matrix in vitro. Their capacity to differentiate into mineralizing cells under this growth condition highlights the potential application of such an approach for future dental tissue engineering strategies. Moreover, our in vitro study provides a culture model for investigating the interactions between epithelial signals and dental stem cells.

Although DSPP, DSP, and DPP have been implicated as markers that are specific for odontoblast differentiation, the DSPP gene coding both DSP and DPP has been detected in dentin, bone, and their respective source cells; in dental pulp cells under inflammatory conditions; and even in the inner ears. Thus, the only objective criterion with which to judge odontoblast differentiation should be the formation of tubular matrix or dentin. Our in vitro findings present direct evidence that induced DPSC pellets are able to form a typical dentin-pulp complex containing clear dentinal tubules and predentin. Previous studies have also regenerated dentin-pulp–like complexes by using DPSCs mixed with hydroxyapatite-tricalcium phosphate powder. However, the outline of these dentin-pulp–like complexes reported elsewhere is irregular, while the shape requirement is much more important for tissue engineering and future clinical applications. Meanwhile, because of the existence of artificial scaffolds, the odontogenic microenvironment is changed and the cell-cell interactions are interrupted for lack of direct cell contact, which are a prerequisite to dental histomorphogenesis. In this study, the formation of dentin-pulp structures with a regular outline suggests that some induced stem cells can achieve commitment and specific competence, allowing them to respond to epithelially derived inductive signals residing in TGC-CM, and differentiate into odontoblast lineage in vitro. During the induction process, DPSCs acquire the ability for morphogenesis from epithelial signals. Under proper conditions, they can reexhibit the developmental events of primary dentinogenesis and mesenchymal morphogenesis. The results also indicate that the genetic information for morphogenesis generated by epithelial signals may be reserved in every induced cell.

Transplantation studies have demonstrated that dental pulp cells can differentiate into chondrocytes and osteoblasts secreting cartilage-like and bone-like matrices. In the current study, 3-dimensional pellet culture in vivo can promote the differentiation and mineralization of DPSCs. Because of the absence of epithelial signals, untreated DPSCs cannot obtain the genetic information for morphogenesis. When
transplanted into the renal capsules, the DPSCs in pellets differentiate into matrix-forming cells at a less coordinated pace, trap themselves in the fast-deposited matrix, and ultimately result in the formation of bone-like tissues with distinct osteoblast-like cells and osteocyte-like lacunae, which are consistent with results reported by other investigators.8,22,29,37 Our observations suggest that DPSCs would be a potential source for bone tissue engineering since the difference between dentin and bone is largely due to a difference in the matrix structure.33 These in vivo results highlight a promising approach of a hybrid tooth-bone tissue engineering using DPSCs and induced DPSCs for the eventual clinical treatment of tooth loss accompanying alveolar bone resorption.

In conclusion, the data reported here indicate that the cocktail of soluble factors containing epithelial environment, which promotes DPSCs to differentiate into functional odontoblasts and form the regular-shaped dentin-pulp complex. These soluble factors, especially growth factors, which are expressed by TGCs (e.g., TGF-β1, TGF-β3, BMP-2, BMP-4, BMP-6, BMP-7, IGF-1, IGF-11, aFGF, and bFGF37), may exert a temporospatial effect on the primary dentinogenesis, particularly on the initiation of odontoblast differentiation. However, it is very difficult to identify the specific roles of these growth factors during development because these molecules act as both paracrine and autocrine factors, several of these molecules are biologically redundant, and synergistic effects exist.37 A long-time observation is required to elucidate the further impact of these factors in TGC-CM. Furthermore, whether DPSCs co-cultured with TGC-CM from different tooth germ cells can bring about the bioengineered dentin-pulp complex with different shapes and whether this conditioned medium has the same inductive effect on other type of stem cells still need careful investigation.

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DENTAL PULP STEM CELLS DIFFERENTIATED INTO DENTIN-PULP COMPLEX


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