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Cell pellets from dental papillae can reexhibit dental morphogenesis and dentinogenesis

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Abstract

We isolated dental papilla mesenchymal cells (DPMCs) from different rat incisor germs at the late bell stage and incubated them as cell pellets in polypropylene tubes. In vitro pellet culture of DPMCs presented several crucial characteristics of odontoblasts, as indicated by accelerated mineralization, positive immunostaining for dentin sialophosphoprotein and dentin matrix protein 1, and expression of dentin sialophosphoprotein mRNA. The allotransplantation of these pellets into renal capsules was also performed. Despite the absence of dental epithelial components, dissociated DPMCs with a complete loss of positional information rapidly underwent dentinogenesis and morphogenesis, and formed a cusp-like dentin-pulp complex containing distinctive odontoblasts, predentin, dentin, and dentinal tubules. These results imply that DPMCs at the late bell stage can reexhibit the dental morphogenesis and dentinogenesis by themselves, and epithelial–mesenchymal interactions at this stage may not be indispensable. Furthermore, different DPMC populations from the similar stage may keep the same developmental pattern.

Keywords: Dental papilla; Morphogenesis; Dentinogenesis; Epithelial–mesenchymal interaction; Tissue engineering; Positional information; Pellet culture; Odontoblast differentiation

Epithelial–mesenchymal interactions are necessary for cell differentiation, tissue regeneration, and morphogenesis in various organs [1–4]. Tooth develops as an ectodermal organ, in which sequential and reciprocal epithelial–mesenchymal interactions govern the tooth initiation, morphogenesis, primary dentinogenesis, and cell differentiation through several signaling pathways [5–9]. At the beginning of odontogenesis, the ectomesenchyme seems to supply the initial inductive signals, which are followed by the formation of dental placode. Subsequent cell proliferation, condensation, polarization, and differentiation of the epithelium and mesenchyme contribute to the tooth morphogenesis [2,9]. Tissue recombination studies have provided evidences that embryonic oral epithelium can elicit odontogenic responses and result in tooth-like morphogenesis both in neural crest-derived mesenchyme and in non-dental cell-derived mesenchyme that does not normally form teeth [10,11]. Together, at the early stages of tooth development, there is no doubt that the tooth morphogenesis and primary dentinogenesis are controlled by the interactions between dental epithelium and mesenchyme.

As we all know, when the first layer of predentin matrix is produced between dental epithelial and mesenchymal cells at the following developmental stages, the reciprocal interactions will disappear because of the physical barrier, indicating that the epithelial–mesenchymal interactions are

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transient events during the odontogenesis process. However, there are few investigations and little knowledge about when and how the reciprocal interactions fade out during the tooth development. Additionally, at the adult stage, although the functional odontoblasts consistently secrete dentin matrix around the dental pulp throughout the life of a healthy tooth, the dental mesenchyme in the pulp cavity still maintains the normal morphology. It seems that the mesenchyme itself may carry the genetic information for morphogenesis which contributes to the maintenance of a normal pulp cavity. Many studies have shown that, at the cap stage, the dental mesenchyme which regulates tooth shape has acquired the odontogenic capacity to instruct tooth morphogenesis [2,6,12–15]. Recently, in vitro experiment further demonstrates that the cap-stage mesenchyme can induce dissociated dental epithelial cells to restore a complete histogenesis of an enamel organ [16]. Although reaggregated dental papilla mesenchymal cells (DPMCs) can produce a tooth when combined with intact dental epithelium [15], it is still unclear whether these DPMCs at the late bell stage can reexhibit the mesenchymal morphogenesis by themselves in the absence of dental epithelial elements and basement membrane.

Taken into consideration that stem cells are capable of self-renewal and multi-lineage differentiation, which make them very promising in regenerating organs and tissues, these cells are of paramount importance in dental morphogenesis [9,17]. Since the putative stem/progenitor cells residing in the dental papillae can differentiate into odontoblasts and perform dentinogenesis without epithelial–mesenchymal interactions [18–20], it seems that these cells may also hold the potential to carry out the dental morphogenesis and make a dentin-pulp complex under appropriate circumstances.

In this paper, our study was designed to evaluate whether the mesenchymal morphogenesis, dentinogenesis, and developmental pattern of DPMCs at the late bell stage are dominated by themselves. We separated the dental mesenchyme from the epithelium/basement membrane and further dissociated the mesenchyme to obtain pure DPMCs. Then, we compared the characteristics of DPMC pellet cultures with monolayer cultures in vitro and investigated the development of DPMC pellets in vivo.

Materials and methods

Cell isolation and culture. Twenty incisor germs were carefully isolated from neonatal Sprague–Dawley (SD) rat pups using dental explorer. The dissected intact lower and upper incisors (Fig. 1A) at late bell stage were treated with 2% collagenase–Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL) at 37 °C for 10 min (Fig. 1B). Dental papillae (Fig. 2B) were physically separated from enamel organs (Fig. 1C) and apical buds (Figs. 1D and 2A) containing dental epithelial cells under a stereomicroscope (Leica MZ9.5, Leica Microsystèms, Germany). The DPMCs were enzymatically obtained from dental papillae (Fig. 2B). First, the dental papillae were minced into <1-mm-sized pieces in 0.01 M phosphate-buffered saline (PBS; Gibco-BRL, Bethesda, MD) and digested with type I collagenase (0.66 mg/mL; Sigma, St. Louis, MO) for 40 min. Subsequently, cells were dissociated by gentle trituration, collected by centrifugation, and washed twice in DMEM (Gibco-BRL) containing 10% fetal bovine serum (FBS), 0.292 mg/mL Glutamax, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 2.5 μg/mL ascorbic acid. Finally, single cell suspensions were generated by filtration through a 70-μm strainer, washed again with DMEM (Gibco-BRL) supplemented with 10% FBS, and then placed into 75 cm^2 culture flasks (Costar, Cambridge, MA, USA) at 1×10^5 cells/mL and cultured in 5% CO_2 at 37 °C. Cells were routinely observed under phase-contrast inverted microscope (Olympus Optical Co. Ltd., Japan) to evaluate their appearance. No epithelial cells were observed by microscopy in the primary cultures. The mesenchymal nature was further confirmed by the negative immunostaining for cytokeratin (Fig. 2C) and positive staining for vimentin (Fig. 2D), the putative epithelial and mesenchymal cell markers [21], respectively (polyclonal antibodies; Sigma, St. Louis, MO).

Three-dimensional pellet culture. DPMCs from the secondary passage were harvested by exposure to 0.25% trypsin/EDTA (Life Technologies, Inc.) for 4 min at 37 °C, washed twice with DMEM and 2 mL medium containing 1×10^6 cells and centrifuged in a 10-mL conical polypropylene tube (Asahi Techno Glass Corp., Tokyo, Japan) at 800*g* for 5 min. Pellets were maintained in tubes and incubated in DMEM supplemented with 20% FBS at 37 °C. The medium was changed every 24 h. For comparison, a traditional monolayer culture was performed at a density of 1×10^5 cells/mL in a 90-mm dish. Generally, the experiment for pellet culture was repeated 45 times in this study. Pellets and monolayer cells at day 1, 3, and 7 were fixed with 4% polyoxymethylene and processed for routine hematoxylin and eosin (H&E) staining.

Alkaline phosphatase (ALP) activity. To evaluate the ALP activity in monolayer cells and pellets with culture time, DPMCs were seeded at a density of 4×10^4 cells/well in 24-well plates or incubated as a pellet with 3×10^5 cells in tubes. After 1, 3, 7, 9, and 14 days of culture, the ALP activity in monolayer DPMCs and cell pellets was detected using ALP assay kit (JianCheng Co., Nanjing, China) according to the manufacturer's instructions. The results were measured spectrophotometrically at 520 nm and described by King and Armstrong units [22].

Von Kossa staining. When mineralization was detected in monolayer cells at day 21 and in pellets at day 3, the samples were fixed with 4% polyoxymethylene and von Kossa staining was then performed. First, monolayer cells and pellet sections were incubated with 5% silver nitrate in dark for 30 min at room temperature. Second, samples were rinsed twice with ddH_2O, exposed to ultraviolet light for 1 h until color development was complete, and then immersed in 5% sodium thiosulfate solution for 2 min. Finally, all samples were counterstained with nuclear fast red solution before visualization by microscopy. All figures for mineralization stained in dark brown or black are typical results of three separate experiments.

Immunocytochemical analyses. Pellets and monolayer cells at day 3 were fixed with 4% polyoxymethylene. Pellets were further embedded in paraffin and sectioned at 5-μm intervals. Then, immunocytochemical evaluation was performed using the streptavidin–biotin complex (SABC) method according to the manufacturer's recommended protocol. Polyclonal antibodies included: rabbit anti-rat dentin sialophosphoprotein (DSPP) at 1:50 dilution (gift from Dr. Fisher, NIDCR, Bethesda, MD) and affinity-purified goat anti-rat dentin matrix protein 1 (DMP 1) at 1:50 dilution (Santa Cruz Biotechnology Inc., Delaware, CA). All samples were counterstained with hematoxylin, and then examined with Olympus compound microscope (Olympus Optical Co. Ltd., Japan).

Reverse transcription-PCR (RT-PCR) for dentin sialophosphoprotein (DSPP) gene. After 3-day culture, DPMC pellets and monolayer cells were harvested. Total cellular RNA was isolated by Trizol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA synthesis and PCR processes were performed as reported [23]. Primer sequences for DSPP (GenBank Accession No. NM012790) and β-actin (GenBank Accession No. NM031144) were as below: (1) DSPP-Sense, 5′-TAAGGACAAGGA CGAATC-3′, and DSPP-Antisense, 5′-ACTGCTGTCACTGCTTTC-3′; and (2) β-actin-Sense, 5′-GAGACCTTCAACACCCCAGCC-3′, and β-actin-Antisense, 5′-CATAGCACAGCTTCTCTTTAA-3′, used as an internal control. The following 30-cycle protocol was used: denaturation at 94 °C for 45 s, annealing at 57 °C for 45 s, extension at 72 °C for 60 s,

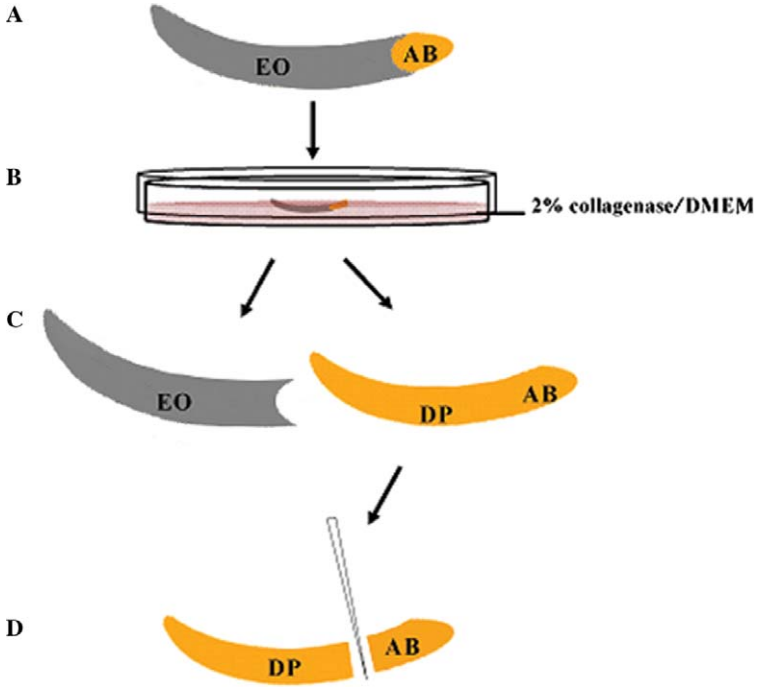

Fig. 1. Schematic diagram for the isolation of dental papillae. (A) Intact incisor germ contains enamel organ (EO), dental papilla (DP), and apical bud (AB). (B) The isolated incisor germs were treated with 2% collagenase–DMEM at 37 °C for 10 min. (C) The dental papilla and enamel organ were physically separated from each other. (D) The apical third of dental papilla involving apical bud and other dental epithelial cells was cut off and discarded in order to get pure mesenchymal tissue.

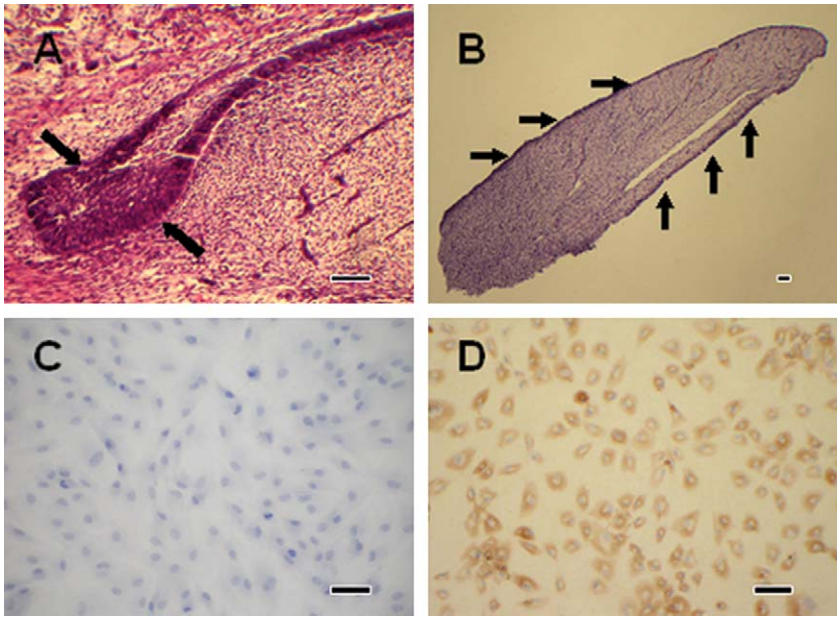

Fig. 2. H&E stained apical bud, dental papilla, and immunocytochemical analyses for primary DPMCs. (A) The apical bud from neonatal incisor germ mainly consists of dental epithelial cells (black arrow). (B) Pure mesenchyme was isolated, around which there were a layer of odontoblasts (black arrows). (C) To exclude the epithelial contamination, the mesenchymal nature of primary DPMCs was confirmed by negative immunostaining for cytokeratin. (D) The mesenchymal nature was further proved by the positive immunostaining for vimentin in DPMCs. Scale bars, 50 μm.

and finally 74 °C for 5 min. The PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and digital images were taken in the ultraviolet background. The expected product sizes from these primers were 472 bp (DSPP) and 283 bp (β-actin), respectively. The experiment was repeated 3 times. PCR products were further confirmed by sequencing (Sangon Biotechnology Co., Shanghai, China).

Transplantation of DPMC pellets. Forty kidneys from 20 adult SD rats were used for allogenic transplantation. Our animal use protocols (rat) were reviewed and approved by the Animal Care Committee of Fourth Military Medical University. DPMC pellet containing 1×10^6 cells, approximately 1.5 mm^3 in size, was incubated in conical tubes at 37 °C for 5 h in order to make them well aggregated, and then seeded directly into

the renal capsules with modified pipette tips on the left side of rat host. As a control, the dental papillae from the lower incisors, which had been separated from enamel organs and apical buds, were implanted into the other side of the same host. Transplantation procedures were performed as previously reported [24]. The environment within the renal capsules supplies the implants with correct physiological conditions and little rejection responses favorable for their sustainable development. Totally we implanted 20 pellets and 22 dental papillae into renal capsules, and the development of these implants was assessed after 14-day incubation.

Analyses of implanted tissues. Excised implants were processed in the serial paraffin sections and analyzed by H&E staining. Immunohistochemical analysis was also performed with the polyclonal rabbit anti-rat ameloblastin (1:400 dilution; gift from Dr. Y. Tian, Fourth Military Medical University, China). In the presence of mesenchymal cells, antibodies against ameloblastin were more accurate than amelogenin for the detection of differentiated ameloblasts [25]. Thus, we used ameloblastin as a marker for detecting the ameloblasts in order to exclude the dental epithelial contamination in experimental and control implants.

Results

Comparison of pellet culture with monolayer culture

Almost all pellets were oval and suspended in conical tubes. The sequential morphological changes in both cultures were examined by H&E staining. Generally, monolayer cells proliferated quickly within 7-day culture time (Fig. 3D–F), while the cell density in pellet cultures decreased gradually and matrix accumulated progressively with culture time (Fig. 3A–C). The nuclei of DPMCs in pellets were mainly oval at day 1 and 3 (Fig. 3A and B). At day 7, almost all cell nuclei in pellets disappeared because of the deposition of homogeneous matrix (Fig. 3C), while there were no apparent changes of nuclei in monolayer cells. More interestingly, at day 3, the peripheral cells of pellets had a tendency to rearrange themselves in a highly ordered way (Fig. 3B). Because of this unusual morphology, we performed further investigations in vitro mainly about the 3-day pellets.

The ALP level in pellet cultures dropped down quickly at first mainly because of the lack of adequate nutrition inside the pellets which could lead to the cell death or apoptosis. After 3 days, the survived cells in pellets began to differentiate and caused a slight increase of ALP activity with culture time (Fig. 4A). The ALP activity in monolayer cells increased quickly after 3 days and reached the peak level at day 7 (Fig. 4A) when cells were almost at the full confluence (Fig. 3F), then decreased gradually because of cell inhibition. ALP is thought to be a marker for odontoblast differentiation, because odontoblasts show much higher ALP activity than dental undifferentiated mesenchymal cells [26]. In pellet cultures, the changes of ALP activity did not reflect the truth of cell differentiation because of the quick mineralization and poor proliferation inside pellets. Hence, ALP should not be used as a marker for odontoblast differentiation in cell pellets in vitro. Mineralization of the dentin matrix is a hallmark of functional and fully differentiated odontoblasts [27]. DPMCs in pellet cultures began to mineralize at day 3 (Fig. 5B), while those in monolayer cultures could not spontaneously form mineralized nodules up to 21-day routine culture (Fig. 5A), as indicated by von Kossa stain.

At day 3, cell pellets were stained positively for cytoplasmic DSPP (Fig. 5C) and DMP 1 (Fig. 5D), respectively, while no staining for DSPP (Fig. 5E) and DMP-1 (Fig. 5F) could be observed in monolayer cells by immunocytochemical technique. Gene expression for DSPP was detected in cell pellets while no traces presented in monolayer cells at day 3 (Fig. 4B).

Collectively, these findings demonstrate that the pellet culture system can stimulate the differentiation of DPMCs

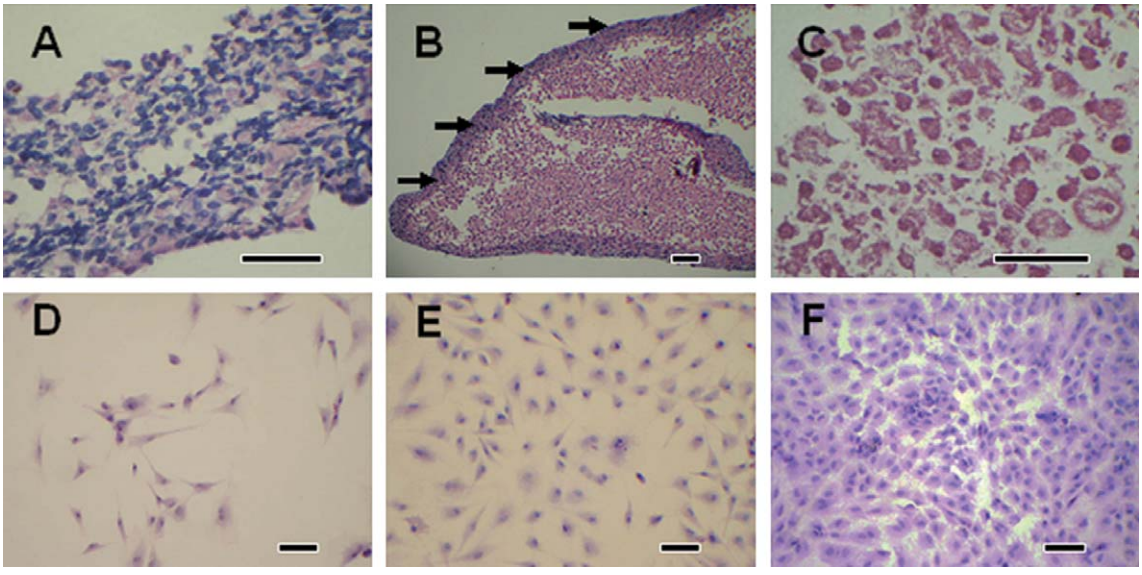

Fig. 3. Morphological changes in the pellet and monolayer cultures (H&E staining). (A) Pellet culture at day 1. (B) At day 3, the peripheral cells in pellet cultures tended to reorganize themselves in a highly ordered way (black arrows). (C) At day 7, all cell nuclei in pellet cultures disappeared because of the matrix deposit. Generally, the cell number decreased gradually with culture time in pellet cultures. (D–F) The monolayer cells at day 1 (D), day 3 (E), and day 7 (F) showed a quick proliferation with culture time, and almost reached the full confluence at day 7. Scale bars, 50 μm.

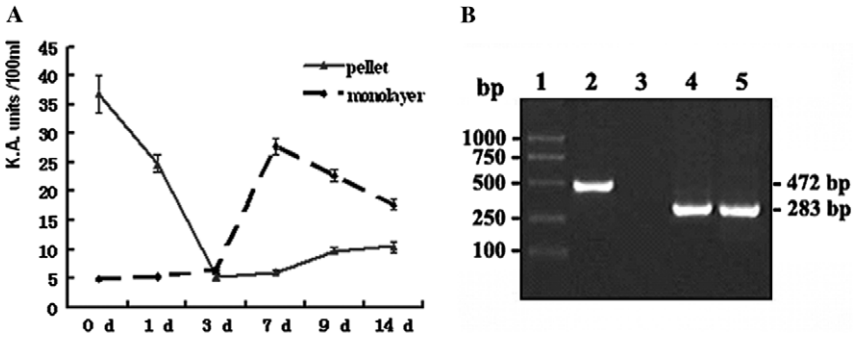

Fig. 4. Comparison of ALP activity and DSPP expression in two different culture systems. (A) The ALP activity in the cell pellet and monolayer culture. Each point is expressed as the mean ± SD of eight determinations. Solid line and dashed line represent pellet and monolayer cultures, respectively. (B) Electrophoresis of RT-PCR products with the 30-cycle protocol: lane 1, DNA markers (bp); lane 2, DSPP in DPMC pellets after 3-day culture; lane 3, DSPP in monolayer cells at day 3; lanes 4 and 5, β-actin was used as an internal control for experimental and control group, respectively.

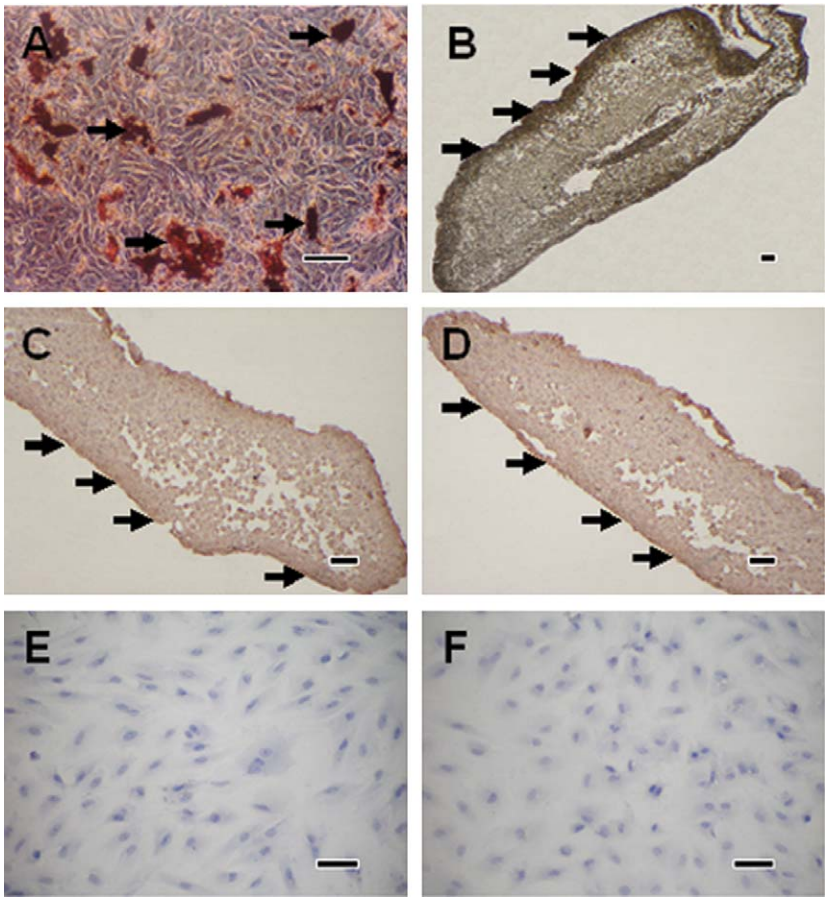

Fig. 5. Von Kossa and immunocytochemical staining in two culture systems. (A) Monolayer cells could not spontaneously form the mineralized nodules up to 21-day routine culture, as indicated by von Kossa stain (black arrows). Dark brown represents a positive appearance for mineralized tissues/nodules. (B) Calcified mineralization was detected in cell pellets at day 3 (black arrows). (C) Cell pellets at day 3 were immunopositive to the DSPP antibody (black arrows). (D) Positive immunostaining for DMP 1 in pellet culture (black arrows). (E) Negative immunostaining for DSPP in monolayer cells. (F) Negative immunostaining for DMP 1 in monolayer cells. Scale bars, 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

into odontoblast lineage without the existence of dental epithelial components. Considering the poor proliferation in pellets in vitro and the requirements of transplantation in vivo, it seems reasonable that the incubation time of cell pellets in vitro should be less than 3 days.

Histological analyses of experimental and control implants

Based on the in vitro experiments, an in vivo evaluation of DPMCs in the rat hosts was performed. Pellet culture in renal capsules has been proved to be a useful model for

studying the development of transplants, in which the resulting tissues are all donor-derived and the host tissues including renal epithelium make no cellular contribution to these bioengineered structures [10,15]. Histological analyses of 2-week implants showed that all control implants developed into accurately formed dentin-pulp complexes containing identifiable dentinal tubules and pulp cavities (Fig. 6C and D). In experimental group, four implants revealed approximately 2 mm × 2 mm (Fig. 6A and B), regular dentin-pulp complexes with a cusp-like shape (Fig. 6E and G), in which the odontoblasts, predentin, dentin, and dental pulp were clearly observed (Fig. 6E and F). At a higher magnification, dentinal tubules inside the predentin and columnar odontoblasts with characteristic cellular processes were visible in the dentin-pulp complex (Fig. 6F). The shape of bioengineered dental tissues is not defined by the pellet outline, which has been proved by previous studies [10,15,16]. Infiltrating lymphocytes were occasion-

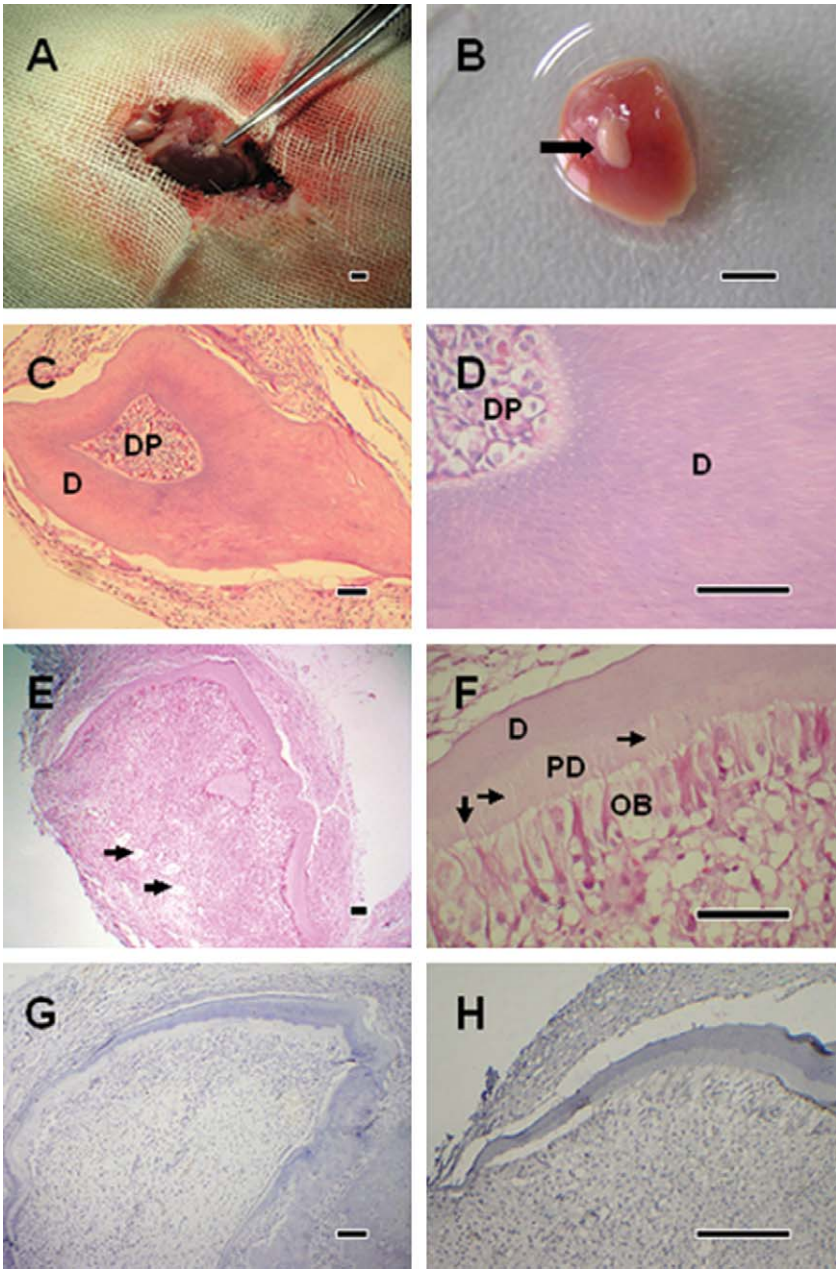

Fig. 6. Histological analyses of control and experimental implants. (A) Image of a kidney containing 14-day-old mineralized pellet implant (indicated by the forceps). (B) The higher magnification of mineralized pellet under the renal capsule (black arrow). (C) The dental papilla implant exhibited a well-formed dentin-pulp complex with a dental pulp cavity (DP). (D) The higher magnification of the tubular dentin (D) involving numerous dentinal tubules in the control implant. (E) Fourteen days pellet sample of DPMCs developed into a distinctive cusp-like dentin-pulp complex with blood vessel-like structures (black arrows). (F) The dentinal tubules (black arrows), predentin (PD), dentin (D), and columnar odontoblasts (OB) are apparent in the dentin-pulp complex. (G) Negative immunostaining for ameloblastin in experimental group. (H) Negative immunostaining for ameloblastin in control group. Scale bars, 2 mm (A,B), 50 μm (C–H).

ally seen in several sectioned implants. Ameloblast-like epithelial cells and enamel structures were not observed in all experimental and control implants, which were further confirmed by negative immunostaining for ameloblastin (Fig. 6G and H).

In general, the pellet culture in vivo creates the most odontogenic microenvironment which can promote the self-differentiation of DPMCs into functional odontoblasts and form the typical dentin-pulp complex with a cusp-like shape.

Discussion

Many studies have proved that tooth-specific tissues involving dentin and pulp can be engineered from tooth germ cells and adult stem cells [9,28]. Our data further demonstrate that reaggregated DPMCs can perform the specific morphogenesis of cusp-like dentin-pulp complex without the induction of dental epithelium, suggesting that the fate of DPMCs at the late bell stage is dominated by themselves and odontogenic information from dental epithelium may be held in reserve in every single mesenchymal cell.

Intercellular communications through growth factors and extracellular matrix (ECM) are believed to play central roles in the spatiotemporal regulation of tooth morphogenesis and dentinogenesis [8,9,29–32]. Up to now, many growth factors, which act as cell-signaling molecules, including TGFs (transforming growth factors), IGFs (insulin-like growth factors), BMPs (bone morphogenetic proteins), FGFs (fibroblast growth factors), PDGFs (platelet-derived growth factors), CTGFs (connective tissue growth factors), EGFs (epidermal growth factors), VEGFs (vascular endothelial growth factors), NGF (nerve growth factor), and TNFs (tumor necrosis factors), have been shown to facilitate various stages of tooth development and provide exciting opportunities for tooth regeneration and engineering [5,9,29,33–35]. Experimentally, dentinogenesis can be induced by certain growth factors or by ECM-absorbed agar substratum [18,19,36]. During tissue morphogenesis, the interactions with ECM can cause cells to directionally migrate, to proliferate, and to differentiate [19,31]. In this study, although original intercellular ECM has lost during cell preparation process, reaggregated mesenchymal cells in pellet culture can secrete new ECM [20,37] and growth factors necessary for tissue formation and regeneration. The presence of ECM, with its endogenous growth factors, may mimic the odontogenic microenvironment created by embryonic basement membrane and inductive signals [8], which is responsible for the recurrence of dentinogenesis and morphogenesis.

Moreover, it is commonly believed that progenitor/stem cells are involved in continuous maintenance and repair of most tissue types. Putative progenitor/stem cells capable of inducing dentinogenesis have been reported in dental pulp/papilla [20,23,38,39]. When covered with embryonic oral epithelium, stem/progenitor cell pellets of non-dental origin cultured in renal capsules can perform odontogenesis and create tooth-like tissues [40]. In this study, it seems that the interactive triad of ECM, bioactive molecules including many growth factors, and progenitor/stem cells inside dental papillae may contribute to the reexhibition of dental morphogenesis and dentinogenesis.

Tooth morphogenesis is a complex event involving serial mitotic activities, apoptosis, cell adhesion, and cell segregation. Since these activities are regulated in time and space, positional information is particularly important. Positional information is defined by gradients of morphogens, cell–cell, and cell–matrix interactions [41]. In this report, although all the pre-existing positional information for mesenchymal cells is completely lost, cells can reorganize themselves into tooth-forming configurations in vivo. The morphogenesis by dissociated DPMCs suggests that mesenchymal cells from different tooth germs at the similar stage may keep the same positional information/developmental pattern during tooth development. This phenomenon is much meaningful in the future applications of tooth engineering because of the complementary action and cytocompatibility between different dental cell populations.

It is well known that odontoblast differentiation and primary dentinogenesis in tooth development depend on the inductive signals derived from the enamel organ and its associated basement membrane [2,6,7,20,36,42]. Our findings show that even if these inductive epithelial signals are lacking, DPMCs at the late bell stage can still fulfill odontoblast differentiation and dentinogenesis, implying that the epithelial–mesenchymal interactions at this stage may not be indispensable.

The pellet culture system, which can provide three-dimensional growth environment necessary for cell differentiation, is helpful to the secretion of ECM which will function as a natural scaffold in pellets [37]. Culture of DPMC pellets in vitro can promote the odontoblast differentiation, as indicated by the accelerated mineralization, positive immunostaining for dentin-specific markers DSPP and DMP 1, along with the expression DSPP mRNA in pellets. A potential reason for these changes may be that cell–cell and cell–matrix interactions in pellet cultures provide an optimal odontogenic microenvironment which is favorable for odontoblast differentiation and maintenance of morphogenetic information. Although these possible spatial and temporal interactions inside the pellets are far from being understood, the pellet culture system provides a valuable model for investigating the differentiation of odontoblasts, the mechanism of dentinogenesis, and the successful bioengineering of artificial teeth.

Traditional tissue engineering approaches usually focus on the injection of isolated cell suspensions or the use of biodegradable scaffolds to support tissue formation. Here, we have developed the ‘cell pellet engineering’, which may allow for tissue regeneration by either transplanting cell pellets in vivo or creating three-dimensional structures in vitro. In this work, DPMC pellet in vitro spontaneously differentiated into odontoblast lineage while in vivo pellet finally formed a regular dentin-pulp complex with a cusp-like shape. When combined with hydroxyapatite (HA)

porous ceramics as previously reported [43], DPMCs differentiate into a bone-like tissue whose shape is much irregular, indicating that the signaling network and cellular homeostasis in DPMC pellets may be different from those in DPMC-ceramic composites, which subsequently cause the differences in cell differentiation and reorganization. The existence of artificial scaffolds may impose negative influence on the cell–cell interactions as well as on the odontogenic microenvironment. Furthermore, host inflammatory responses to these polymer materials are often inevitable. Thus, cell pellet engineering avoids several potential problems associated with biodegradable scaffolds and presents some significant advantages in tooth engineering.

In summary, the data reported here indicate that the mesenchymal cells at the late bell stage, which are completely free from dental epithelium and basement membrane, can re-perform dental morphogenesis and dentinogenesis under suitable circumstances. The DPMCs from different teeth at the similar stage still keep the same developmental pattern during the re-odontogenesis. However, whether DPMCs from earlier stages can reexhibit the dental morphogenesis too by themselves, and what impact the biodegradable scaffolds exert on cell differentiation still need careful investigations.

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