Preliminary Study on Dental Pulp Stem Cell–mediated Pulp Regeneration in Canine Immature Permanent Teeth

Yuanyuan Wang, MD, Yuming Zhao, PhD, Weiqian Jia, MD, Jie Yang, MD, and Lihong Ge, PhD

Abstract

Introduction: The health of human teeth depends on the integrity of the hard tissue and the activity of the pulp and periodontal tissues, which are responsible for nutritional supply. Without the nourishing of the pulp tissue, the possibility of tooth fracture can increase. In immature permanent teeth, root development may be influenced as well. This study explored the potential of using autologous dental pulp stem cells (DPSCs) to achieve pulp regeneration in a canine pulpless model. **Methods:** The establishment of the pulpless animal model involved pulp extirpation and root canal preparation of young permanent incisor teeth in beagles. Autologous DPSCs were obtained from extracted first molars and expanded *ex vivo* to obtain a larger number of cells. The biological characteristics of canine DPSCs (cDPSCs) were analyzed both *in vitro* and *in vivo* by using the same method as used in human DPSCs. cDPSCs were transplanted into the pulpless root canal with Gelfoam as the scaffold, and root development was evaluated by radiographic and histologic analyses. **Results:** cDPSCs with rapid proliferation, multiple differentiation capacity, and development potential were successfully isolated and identified both *in vitro* and *in vivo*. After they were transplanted into the pulpless root canal with Gelfoam as the scaffold, DPSCs were capable of generating pulp-like tissues containing blood vessels and dentin-like tissue. Thickening of the root canal wall was also observed. **Conclusions:** This study demonstrates the feasibility of using stem cell–mediated tissue engineering to realize pulp regeneration in immature teeth. *(J Endod 2013;39:195–201)*

Key Words

Dental pulp stem cells, immature permanent teeth, pulp regeneration, tissue engineering

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The health of teeth depends on the integrity of the hard tissue and the activity of the pulp and periodontal tissues, which are responsible for supplying nutrition to teeth. Lack of nourishment provided by the pulp tissue can increase the risk of tooth fracture. In immature permanent teeth, impaired root development is another outcome associated with lack of nutritional support of the pulp.

For pediatric dentists, the optimal treatment of pulpless immature permanent teeth would involve the regeneration of pulp tissues that promote the continuation of normal root development. Traditional treatment methods for immature teeth with infected or necrotic pulp tissues, such as apexification, result in shorter root lengths and thinner root canal walls compared with normal teeth, thereby leading to poor long-term prognosis. Some recent studies have reported that revascularization (1–3) could control inflammation, as well as thickening the dentin wall and elongating the root length in infected immature teeth. However, most of these studies are case reports, with animal studies showing that newly formed tissues were bone-like or cementum-like in nature (4, 5). Moreover, tooth discoloration (6) and an increased possibility of root canal obturation (7) have been reported after treatment involving revascularization. In theory, whether such therapy works depends on the quantity and quality of the residual dental pulp stem cells (DPSCs) and stem cells from the apical papilla (8). To date, however, no method has been used effectively to assess the existence of DPSCs and stem cells from the apical papilla in the clinical setting, leading to uncertainty regarding proper indications and outcomes associated with revascularization.

In recent years, some groups have tried to use tissue engineering techniques in pulp regeneration, with some promising results. Huang et al (9) reported that pulp-like and dentin-like tissues were formed on subcutaneous root canal implants containing synthetic scaffolds seeded with stem-progenitor cells from apical papilla and dental pulp. By using CD31(−)/CD146(−) side population cells, Iohara et al (10) achieved pulp regeneration in the canine model. Further research was conducted by this group that involved the application of stem/progenitor cells from the bone marrow and adipose tissue, with satisfactory results (11). Kodonas et al (12) seeded swine DPSCs on organic or synthetic scaffolds before implanting them as hybrid root implants in the jaw bones of mini pigs. The results showed the formation of dentin-like structures.

As essential elements in tissue engineering, scaffold materials have shown their importance in supporting newly formed tissue in pulp regeneration studies. Several scaffold materials have been successfully used for dental pulp tissue engineering, including organic collagen scaffolds (13), as well as the nonorganic polymer poly(lactic-co-glycolic acid) (14, 15), polyglycolic acid (15), and poly-L-lactic acid scaffolds (16). However, most of these materials are synthesized and expensive. Therefore, to meet clinical requirements, a simpler and more practical treatment approach is needed to achieve the goal of pulp regeneration in immature teeth via tissue engineering.

The hypothesis of this study is that DPSCs combined with Gelfoam (absorbable gelatin sponge; Xiang'En House Medical Equipment, Nanchang, Jiangxi Province, China) as the scaffold material could mediate dental pulp regeneration in immature teeth.

Materials and Methods

Animals

Three inbred male beagle dogs aged 18–20 weeks and weighing 12–15 kg were obtained from Marshall Biotechnology Co Ltd (Beijing, China). They were housed in

light- and temperature-controlled rooms and allowed access to food and water *ad libitum*. Their care and the experimental procedures used in this study were in accordance with the guidelines of the U.S. National Institutes of Health regarding the care and use of animals for experimental procedures, as well as with the recommendations of the Beijing Administration Rules of Laboratory Animals.

This study was reviewed and approved by the Animal Care and Use Committee of the Medical School of Peking University (no. LA2011-045).

Cell Culture

Normal human impacted third molars with an open apical foramen were collected from an adult (18-year-old man) at the clinic of the Peking University School of Stomatology and used for culturing human DPSCs (hDPSCs). The patient provided written informed consent, and the protocol to obtain extracted teeth was previously approved by the Ethical Committee of the Medical School of Peking University. Three first molars with root development at Nolla stage 8 were extracted under general anesthesia from each of the beagles in the study to separately culture canine DPSCs (cDPSCs). After the tooth surfaces were cleaned, teeth were cut around the cementum-enamel junction with sterilized dental fissure burs to expose the pulp chamber. The pulp tissue was gently separated from the crown and root and subsequently digested in a solution of 3 mg/mL collagenase type I (Sigma-Aldrich, St Louis, MO) and 4 mg/mL dispase (Sigma-Aldrich) for 1 hour at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-mm strainer (Falcon; BD Biosciences, San Jose, CA). Single-cell suspensions ($0.5-1.0 \times 10^3$/well) of both human and canine dental pulp were seeded onto 6-well plates (Costar; Corning Life Sciences, Tewksbury, MA) containing alpha modification of Eagle medium (GIBCO/BRL, Grand Island, NY) supplemented with 15% fetal bovine serum (Hyclone; Thermo Scientific, Logan, UT), 100 U/mL penicillin, and 100 mg/mL streptomycin (Sigma-Aldrich), and incubated at 37°C in 5% CO_2. To assess colony-forming efficiency, day 7 cultures were fixed with 4% formalin and stained with 0.1% toluidine blue. Aggregates of ≥50 cells were scored as colonies, and those colonies arising from hDPSCs and cDPSCs were counted separately.

Human and canine DPSCs (1.0×10^3/well) expanded *ex vivo* were seeded onto five 96-well plates separately. At days 1, 3, and 5 after cell seeding, a cell counting kit-8 assay was carried out and repeated 8 times to evaluate the number of viable cells according to the manufacturer's instructions. Ten microliters of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (Dojindo, Kumamoto, Japan) was added to each well before the culture plate was incubated at 37°C for 4 hours. Absorbance was measured at 450 nm with a microplate reader (ELx808IU; BioTek, Winooski, VT). The mean values of optical density were calculated and statistically analyzed for hDPSCs and cDPSCs separately at 1, 3, and 5 days. The growth curves of cDPSCs and hDPSCs were analyzed by using the SPSS software (version 13.0; SPSS, Chicago, IL).

Immunohistochemical Staining

The cDPSCs were subcultured onto 12-chamber slides ($2.0 \times$ 104/well, second passage) and grown to 80% confluence; then the cells were fixed in 4% paraformaldehyde and blocked with phosphate-buffered saline (PBS) containing 10% normal equine serum at room temperature for 45 minutes. The cells were then incubated with diluted (1:50) primary antibody STRO-1 (monoclonal mouse anti-human STRO-1 antibody; R&D Systems, Inc, Minneapolis, MN) overnight at 4°C, washed with PBS, and subsequently incubated with fluorescein-conjugated secondary goat anti-mouse polyclonal antibody (STAR 87F; AbD Serotec, Raleigh, NC) at room temperature in the dark for 45 minutes. DAPI (4,6-diamino-2-phenyl indole; ZSGB-BIO, Beijing, China) staining was then performed in the dark for 5 minutes. After being washed with PBS, the slides were analyzed by using a fluorescence microscope (Nikon ECLIPSE TS-100; Tokyo, Japan).

The same passage of cDPSCs was subcultured onto 12-chamber slides ($2 \times$ 104/well, second passage) and grown to 80% confluence. The cells were fixed in 4% paraformaldehyde and washed 3 times with PBS. Antibodies to ALP (1:200 dilution) and nestin (1:200 dilution) were obtained from Boster (Wuhan, China). The antibodies were diluted with Tris-buffered saline (TBS) in the ratios indicated above. The cDPSCs were incubated with one of the primary antibodies overnight at 4°C, followed by consecutive incubations with Polymer Helper and poly-peroxidase-anti-goat/rabbit/mouse immunoglobulin G (Polink-2 Plus HRP System Kits; Zhongshan Golden Bridge Biotechnology, Beijing, China). All incubations were followed by at least 3 washes in TBS. The sections were developed with a DAB (3,3'-diaminobenzidine tetrahydrochloride) substrate kit (Zhongshan Golden Bridge Biotechnology), counterstained with hematoxylin, and examined under a light microscope (Nikon ECLIPSE TS-100). In all cases, negative control staining was conducted in parallel by incubating sections with TBS instead of with the primary antibody.

Differentiation of cDPSCs into Multiple Lineages

Mineralization Potential. Cells were seeded onto 24-well plates, grown to 70% confluence, and incubated in differentiation medium containing 10 nmol/L dexamethasone, 10 mmol/L β-glycerolphosphate, 50 mg/mL ascorbate phosphate, 10 nmol/L 1,25-dihydroxyvitamin D3, and 15% fetal bovine serum for 3 weeks. Cultures were fixed in 4% paraformaldehyde and washed in PBS, and the mineralization of the extracellular matrix was detected by staining with 1% alizarin red S (17).

Adipogenic Differentiation

Cells were seeded onto 24-well plates, grown to subconfluence, and incubated in adipogenic medium containing 1 mmol/L dexamethasone, 1 mg/mL insulin, 0.5 mmol/L 3-isobutyl-1-methylxanthine, and 15% fetal bovine serum for 4 weeks. Cells were fixed in 4% paraformaldehyde for 60 minutes and washed with 70% ethanol. Lipid droplets were stained with 2% (w = v) oil red O reagent for 5 minutes and washed with water (18).

Transplantation

Approximately 1.0×10^7 cDPSCs (third passage) were mixed with 40 mg hydroxyapatite ceramic powder before they were transplanted onto the dorsal surfaces of 10-week-old mice with severe combined immunodeficiency (CB-17 scid mice, NIH-bg-nu-xid; Harlan–Sprague–Dawley; Harlan Laboratories, Indianapolis, IN). These procedures were performed in accordance with the specifications of an approved small animal protocol (National Institute of Dental and Craniofacial Research, no. 97–024). The transplants were recovered at 8 weeks after transplantation, fixed with 4% formalin, decalcified with buffered 10% EDTA (pH 8.0), and embedded in paraffin. Sections (5 mm) were deparaffinized and stained with hematoxylin-eosin.

Establishment of a Pulpless Animal Model

Three 18-to 20-week-old beagles, with root development of anterior teeth at the 7–8 Nolla stage, were used to establish the animal model of pulpless immature permanent teeth. The dogs were first given general anesthesia with pentobarbital natrium (30 g/L; Sinopharm Chemical Reagent Co, Ltd, Shanghai, China) via intravenous injection. After sterilization, 4 upper incisors from each dog were used to generate

a pulpless model via pulp chamber exposure with sterilized dental fissure burs, as well as pulp extirpation with barbed broach. The root canals were prepared and shaped adequately. During treatment, root canals were rinsed with 5.25% NaClO (sodium hypochlorite) and 0.9% NaCl (sodium chloride) alternately for 30 minutes before they were dried with sterilized absorbent points.

DPSC-mediated Pulp Regeneration *In Vivo*

The pulpless immature permanent teeth were divided into 3 groups in each canine.

In group 1, cDPSC/Gelfoam group (n = 6), approximately 2.0×10^7 of the expanded third passage autologous cDPSCs combined with Gelfoam were cocultured for 1 hour at 37°C and subsequently transplanted into the root canals. In group 2, Gelfoam group (n = 6), Gelfoam was cultured in the basic medium for 1 hour at 37°C and subsequently transplanted into the root canals. In group 3, cDPSC group (n = 6), approximately 2.0×10^7 of the expanded third passage autologous cDPSCs were transplanted into the root canals.

For all teeth involved in the experiments, a blood clot was produced at the level of the cementum-enamel junction to provide a nutritious environment for the ingrowth of new tissue, followed by a seal of mineral trioxide aggregate in the cervical area and a bonded resin (Z250; 3M ESPE, St Paul, MN) coronal restoration above it.

Clinical and Radiographic Evaluations

Clinical assessment, including evaluations of tooth mobility, the gingiva, and restoration retention, was carried out every 2 weeks after the operation. Root development, dentinal deposition, and periapical lesions were evaluated by radiography (Focus; Instrumentarium Imaging, Milwaukee, WI) every 4 weeks. All periapical radiographs were taken by the same technician, who used the bisecting angle technique with a projection angle of −37° and an exposure time of 0.1 second. The completion of root development was defined as the closure of the apical foramen and the thickening of the root canal wall. The length of the root, the thickening of the dentin wall, and the width of the apical foramen were measured on the radiographs by 2 different dentists who were trained and who passed the consistency test (kappa value > 0.8) (Fig. 1).

Histologic Assessment of the Regenerated Pulp Tissue

When any of the experimental teeth displayed complete root development, all experimental teeth in the same dog were extracted and fixed in 4% paraformaldehyde, decalcified with buffered 10% dilute hydrochloric acid, and embedded in paraffin. For histologic assessment, serial 5-μm longitudinal sections of the experimental teeth were cut and stained with hematoxylin-eosin.

Statistical Analysis

The Student's *t* test was used to compare the colony-forming efficiency of hDPSCs and cDPSCs.

Differences in root length, foramen width, and wall thickness at each time point among the 3 groups were analyzed by using one-way analysis of variance. Pairwise comparisons were analyzed by using the Bonferroni method.

SPSS software was used (version 13.0), and differences were considered statistically significant at $P < .05$.

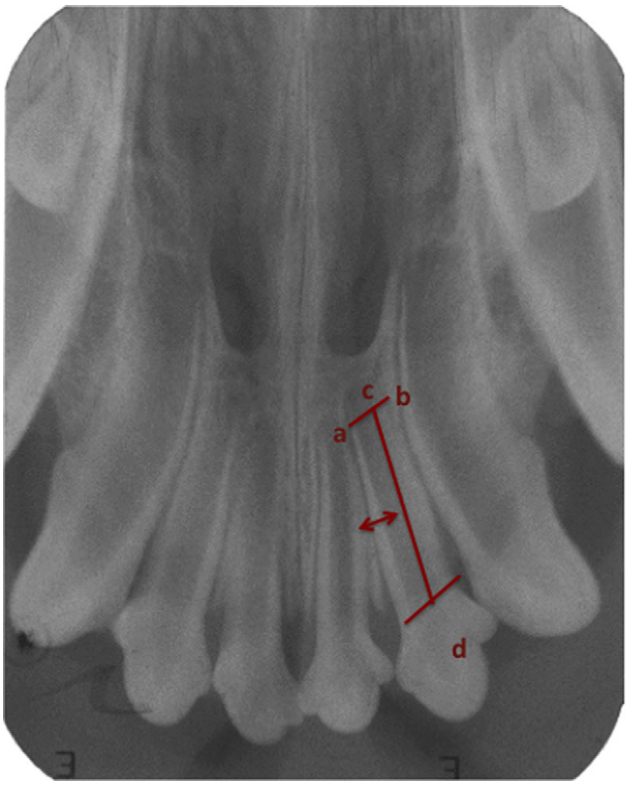

Figure 1. Radiographic evaluation for pulp regeneration. The width of the apical foramen was defined as the distance from "a" (the mesial apical point) to "b" (the distal apical point). The length of the root was defined as the distance from "c" (the midpoint of the line connecting "a" and "b") to "d" (the midpoint of the cervical line). The thickness of the root wall was defined as the thickness of the mesial wall at the midpoint of the root (the midpoint of the line connecting "c" and "d", as indicated by the *arrow*).

Results

Characteristics of the cDPSCs

Cell Isolation and Basic Analysis. The cells within each colony were characterized by a typical fibroblast-like morphology (Fig. 2*A* and *B*). The frequency of the colony-forming cells derived from canine dental pulp tissue (average, 150 colonies/10^4 cells) was significantly higher than that of the colony-forming cells from hDPSCs (average, 60 colonies/10^4 cells) ($P < .05$). The cell counting kit-8 assay showed that cDPSCs had a higher proliferation rate than did hDPSCs (Fig. 2*C*).

Characterization of cDPSCs *In Vitro*

The progeny of the colony-forming cells showed positive immunostaining for mesenchymal stem cell markers such as STRO-1 (Fig. 2*D*), ALP (Fig. 2*E*), and nestin (Fig. 2*F*).

In addition, the cDPSCs had the ability to differentiate into multiple lineages. After 3 weeks of induction, condensed nodules positive for alizarin red S and oil red O–positive lipid droplets were formed (Fig. 2*G* and *H*).

Characterization of cDPSCs *Ex Vivo*

To study their developmental potential, the cDPSCs in conjunction with hydroxyapatite powder were transplanted into immunocompromised mice. At 6 weeks after transplantation, the cDPSCs generated a dentin-like structure, which was composed of a highly ordered

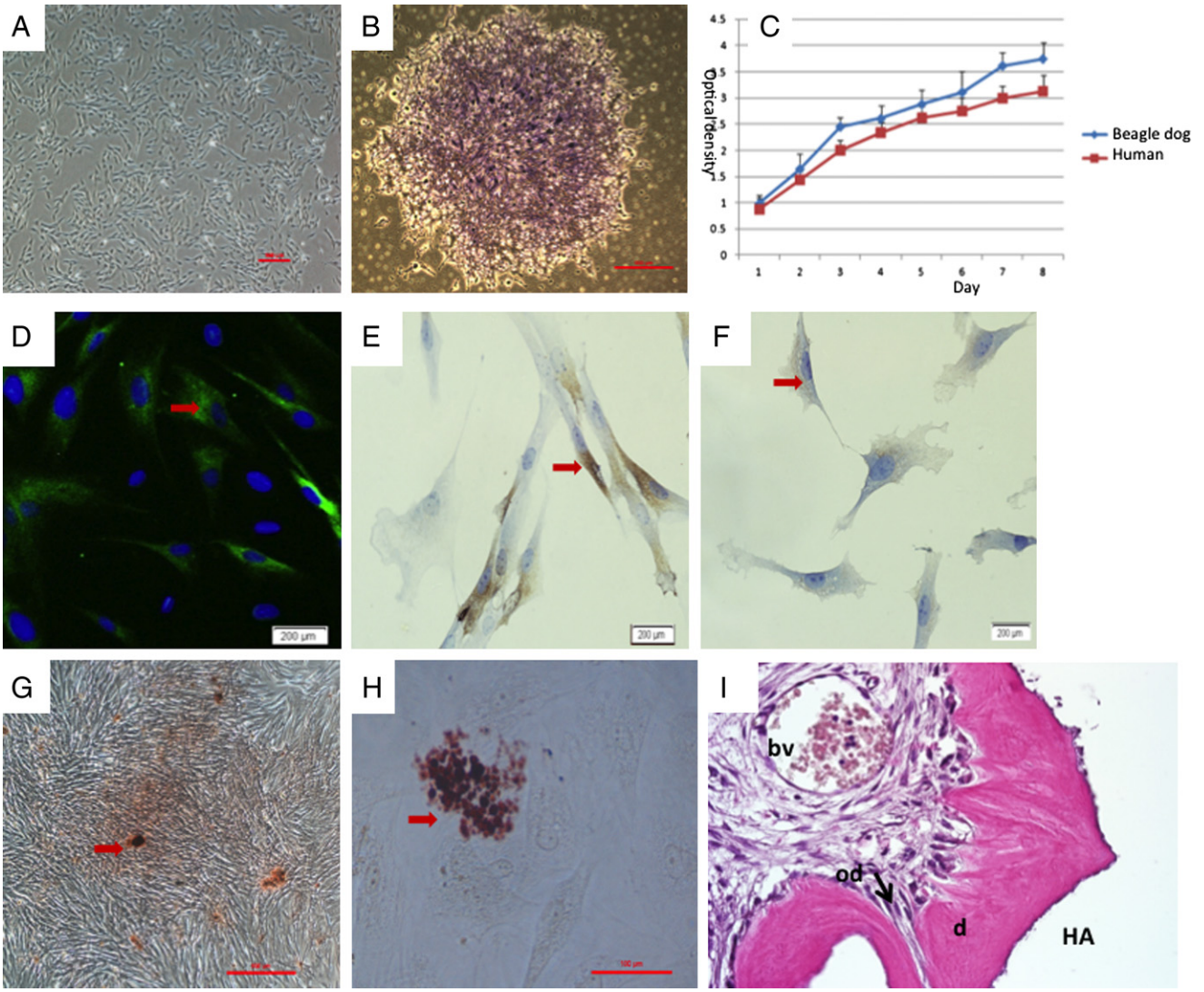

Figure 2. The isolation and biological characterization of beagle DPSCs. cDPSCs have typical fibroblast-like cell morphology (*A*). Representative colonies are formed after culturing for 7 days (*B*). Growth rate of cDPSCs is higher than that of hDPSCs, as shown by CCK-8 assay (*C*). cDPSCs showed positive staining of STRO-1 (*D*), ALP (*E*), and nestin (*F*); the typical positive staining is indicated by the *arrows*. cDPSCs have the ability to differentiate into multiple lineages. They could be induced to form mineralized nodules and lipid droplets, as indicated by *arrows* in (*G*) and (*H*), respectively. Developmental potential of cDPSCs *ex vivo*. Cross section of cDPSC transplants (*I*), 8 weeks after transplantation and stained with hematoxylin-eosin. The hydroxyapatite carrier surfaces are lined with a dentin-like matrix (d), surrounding pulp-like tissue with blood vessels (bv) and an interface layer of odontoblast-like (od) cells (*I*). CCK-8, cell counting kit-8; HA, hydroxyapatite.

collagenous matrix deposited perpendicular to the odontoblast-like layer when viewed on the surface of the hydroxyapatite particles. The odontoblast-like cells displayed protruding cytoplasmic processes into the dentinal matrix, which interfaced with a pulp-like interstitial tissue infiltrated with blood vessels (Fig. 2*I*).

cDPSC-mediated Pulp Regeneration in Canine Immature Teeth

Autologous cDPSCs combined with Gelfoam were transplanted into the root canals of the pulpless immature permanent canines. In this study, clinical examination, periapical radiography analysis, and

TABLE 1. Clinical Evaluation of Root Development in the 3 Groups

	Clinical assessment (24 weeks)			Radiologic evaluation (24 weeks)		
Group (no. of teeth)	Tooth mobility	Gingiva evaluation (swelling and fistula)	Restoration retention	Complete root development	Root wall thickening	Foramen closure
Group 1 (n = 6)	No	No	Good	6	6	6
Group 2 (n = 6)	No	No	Good	4	0	0
Group 3 (n = 6)	No	No	Good	0	0	0

Group 1, cDPSC/Gelfoam group; group 2, Gelfoam group; group 3, cDPSC group.

TABLE 2. Radiographic Analysis of Pulp Regeneration in the Different Groups

	Pretransplantation			24 Weeks		
Group (no. of teeth)	RL	FW	WT	RL	FW	WT
Group 1 (n = 6)	15.3 ± 0.09	1.51 ± 0.09	1.0 ± 0.07	18.7 ± 0.27	0.30 ± 0.07	3.0 ± 0.35
Group 2 (n = 6)	15.4 ± 0.09	1.48 ± 0.08	0.95 ± 0.07	15.6 ± 0.22	1.10 ± 0.07	1.0 ± 0.07
Group 3 (n = 6)	15.3 ± 0.09	1.55 ± 0.07	1.05 ± 0.06	15.5 ± 0.23	1.45 ± 0.07	1.08 ± 0.09

FW, foramen width; RL, root length; WT, wall thickness.

Data are expressed as mean ± standard deviation. Differences of RL at each time point among the 3 groups were analyzed with one-way analysis of variance. Pairwise comparisons were analyzed by using the Bonferroni method.

histologic staining were used to evaluate cDPSC-mediated pulp regeneration.

A clinical examination showed that no tooth mobility was observed in the cDPSC/Gelfoam group or in the Gelfoam group, whereas grade I–II mobility was observed in 2 teeth in the cDPSC group. A fistula was present in 1 of the 2 mobile teeth. Restoration failure was not observed in any of the 3 groups (Table 1).

A radiologic analysis showed that all teeth in the cDPSC/Gelfoam group, 4 in the Gelfoam group, and none in the cDPSC group achieved complete root development, including continuity of root length and closure of the apical foramen, whereas the thickening of the dentinal wall was observed only in the cDPSC/Gelfoam group at approximately 24 weeks after the operation. The results of the statistical analysis showed that before the operation, there was no significant difference among the 3 groups in root length ($F = 2.500$, $P = .116$), foramen width ($F = 0.983$, $P = .413$), and wall thickness ($F = 3.000$, $P = .08$). At 24 weeks after transplantation, the difference in root length between the cDPSC/Gelfoam group and the other 2 groups was statistically significant ($F = 331.811$, $P = .000$); the results of pairwise comparisons at this juncture were as follows: cDPSC/Gelfoam group and cDPSC group, $P = .000$; cDPSC/Gelfoam group and Gelfoam group, $P = .000$; and cDPSC group and Gelfoam group, $P = 1.000$. The difference in foramen width between the cDPSC/Gelfoam group and the other 2 groups was statistically significant ($F = 331.811$, $P = .000$), as was the difference in the root wall thickness ($F = 166.166$, $P = .000$) (Table 2; Fig. 3*A* and *B*).

A histologic evaluation showed that in the cDPSC/Gelfoam group, new dentin was deposited along the radicular inner wall and that the newly regenerated pulp tissue contained blood vessels and dentin-like tissues (Fig. 4*A* and *B*). Some fiber-like tissue was generated in the Gelfoam group, whereas no obvious histologic structures were formed in the cDPSC group, and no dentin-like tissue or blood vessels were observed in either the Gelfoam or the cDPSC groups (Fig. 4*C* and *D*).

Discussion

This study evaluated whether the transplantation of autologous cDPSCs with scaffolds into pulpless root canals could mediate pulp regeneration and root development in canine immature teeth. To evaluate the effect of these cells and scaffolds in pulp regeneration, teeth transplanted with either the DPSCs or scaffold alone were used as controls.

Canines are considered ideal animals for dental research because of the following characteristics: similar growth patterns and pathophysiology to those of humans and easy manipulation. Therefore, we used canines to establish pulpless young permanent tooth models for the study.

On the basis of tissue engineering theory, the first step in tissue regeneration is to obtain appropriate stem cells. In this study, the cDPSCs were isolated and characterized for their mesenchymal stem cell origin. One of the criteria for identifying stem cells is the expression

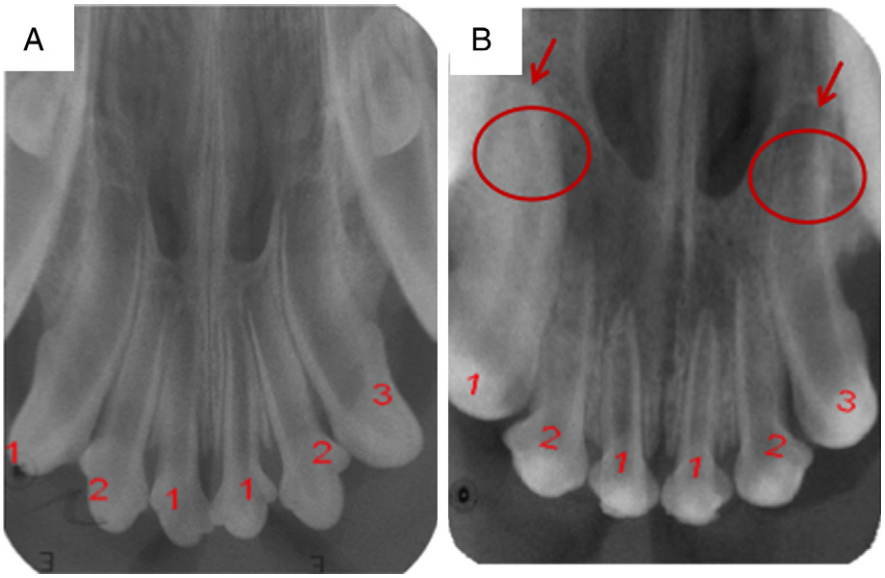

Figure 3. Preoperative radiographs show that upper incisors of 18-week-old canine were immature, with open apical foramina and thin dentin walls (*A*); 24 weeks after the operation, the development of roots was complete in group 1, apical foramen was closed, and dentin wall was thickened. Similar findings were not observed in groups 2 and 3, as indicated by *arrows* (*B*).

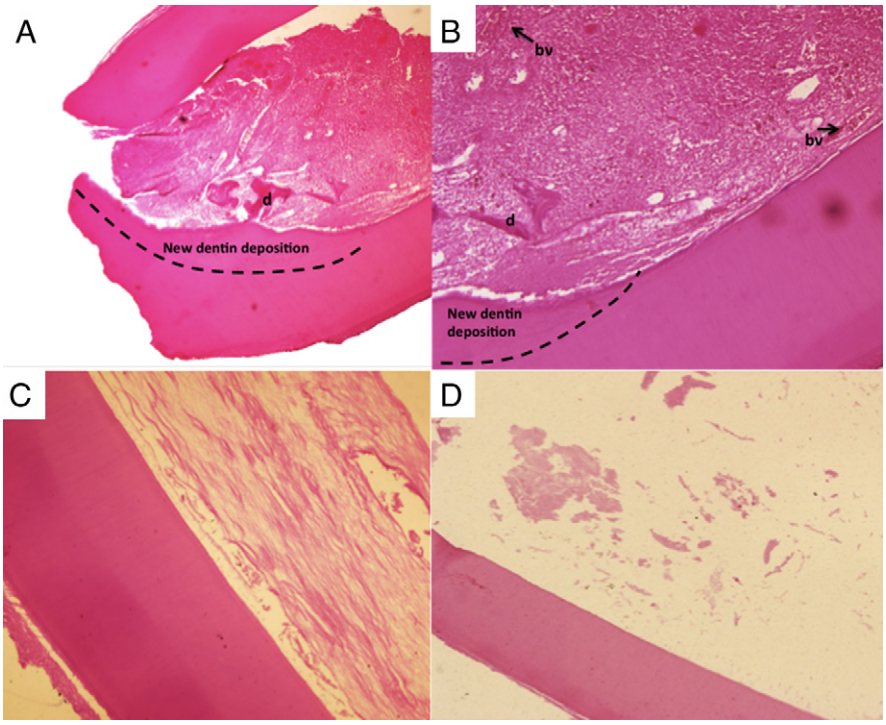

Figure 4. Histologic assessment of pulp tissue regeneration by hematoxylin-eosin staining. In group 1, new dentin was deposited along the radicular inner wall (*A*) in 4 of the 6 teeth. Newly generated pulp tissue contained blood vessels (bv) and dentin-like (d) tissue (*B*). In group 2, some fiber-like tissue was generated within the root canal of 5 teeth (*C*). No obvious histologic structures were observed in all teeth in group 3 (*D*).

of stem cell markers. Previous studies (19) suggested that STRO-1 was a marker for the perivascular niche of these stem cell populations. Recent studies have shown that blood vessel walls harbor a reserve of progenitor cells that might be integral to the origin of mesenchymal stem cells and other related adult stem cells (20). In our study, the isolated cDPSCs were positive for STRO-1 staining. Similar to hDPSCs, the cDPSCs also express other mesenchymal stem cell markers such as ALP and nestin.

Previous experiments have shown that an important feature of the DPSCs is their odontoblastic differentiation potential (18). In early research with DPSCs, hDPSCs showed the *ex vivo* potential for self-renewal and differentiation into osteoblasts, adipocytes, chondrocytes, and neurons; transplantation of hDPSCs mixed with hydroxyapatite/tricalcium phosphate formed an ectopic pulp-dentin–like tissue complex in nude mice (18). Our studies on multilineage differentiation ability showed that the cDPSCs had both mineralization and adipogenesis potential. The formation of a pulp-dentin–like complex in immunocompromised mice suggested that these cells have odontoblastic differentiation potential.

Another key component in tissue engineering is the scaffold (21, 22). Scaffolds have been used in tissue regeneration to facilitate the formation and maturation of new tissues or organs, where a balance between temporary mechanical support and mass transport (eg, degradation and cell growth) is ideally achieved. Since the discovery of hDPSCs, the regeneration of dentin-pulp complex has been mediated by using different scaffolds such as hydroxyapatite/tricalcium phosphate ceramic powder (23), hydrogels (24, 25), synthetic scaffolds (13–15), ceramic scaffolds (26), organic scaffolds (27), and platelet-rich plasma (28). However, none of these scaffold materials is ideal for pulp regeneration, because they are either not suitable for soft tissue regeneration or are too complicated for synthesis and manipulation. Gelfoam is one of the most commonly used dressings for the promotion of wound healing after tooth extraction. As reported, Gelfoam has good biocompatibility and can promote hemostasis and fibrosis (29, 30). Previous reports have suggested that Gelfoam could be used as a scaffold material in soft tissue regeneration in, for example, the reconstruction of a ventricular and perivascular endothelium and meniscus (31–33). Because it is commercially available at quite reasonable prices and easy to manipulate, we used Gelfoam as the scaffold material in our study.

Our *in vivo* studies showed that autologous cDPSCs combined with Gelfoam transplanted into pulpless root canals led to pulp regeneration, thereby resulting in root elongation and dentinal deposition. Furthermore, a histologic analysis showed that these cells demonstrated an excellent capacity to form dentin and blood vessels, which were quite different from the generated tissues formed by revascularization *in vivo*. Similar regeneration results were not seen in the other 2 control groups, supporting the concept of using DPSCs together with scaffolds as a potential way to treat immature teeth with pulp diseases. Further studies are required to understand the mechanisms of DPSC-mediated pulp regeneration and to explore strategies for enhancing pulp tissue regeneration, including the application of growth factors and cytokines.

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The authors deny any conflicts of interest related to this study.

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