Published in final edited form as:

J Endod. 2011 September ; 37(9): 1217–1224. doi:10.1016/j.joen.2011.05.022.

Cells Isolated from Inflamed Periapical Tissue Express Mesenchymal Stem Cell Markers and Are Highly Osteogenic

James Liao, BS^{*}, Mohammed Al Shahrani, BDS^{*}, Mey Al-Habib, BDS^{*}, Toshinori Tanaka, DDS[†], and George T.-J. Huang, DDS, MSD, DSc^{*,†}

^{*}Department of Endodontics, Henry M. Goldman School of Dental Medicine, Boston University, Boston, Massachusetts

[†]Section of Oral and Diagnostic Sciences, Division of Endodontics, College of Dental Medicine, Columbia University, New York, New York.

Abstract

Introduction—We previously reported the presence of mesenchymal stem/progenitor cells (MSCs) in inflamed pulp tissue. Here we asked whether MSCs also exist in inflamed periapical tissues resulting from endodontic infection. The objectives of this study were to detect the expression of MSC markers in periapical inflammatory tissues and to characterize isolated cells from these tissues.

Methods—Human periapical inflammatory tissues were collected and processed to detect MSC marker expression by immunohistochemistry. Cells were isolated and tested for cell surface marker expression by using flow cytometry and examined for multiple differentiation potential into osteogenic and adipogenic pathways. *In vivo* formation of mineralized tissues was assessed in a mouse model.

Results—Immunohistochemistry showed positive staining for MSC markers STRO-1, CD90, and CD146. Isolated cells at passage 0 appeared as typical fibroblastic cells, and a few cells formed colony-forming unit-fibroblasts (CFU-Fs). After passaging, the CFU-F forming ability diminished dramatically, and the population doubling was up to 26. Flow cytometry data showed that these cells at passage 2 expressed low levels of STRO-1 and CD146 and moderate to high levels of CD90, CD73, and CD105. At passage 6, the levels of these markers decreased. When incubated in specific differentiation medium, cells demonstrated a strong osteogenic but weak adipogenic capacity. After *in vivo* cell transplantation, mineralized tissues formed in immunocompromised mice.

Conclusions—Human periapical inflammatory tissues expressed MSC markers, suggesting the presence of MSCs. Isolated cells exhibited typical mesenchymal cell immunophenotype with a capacity to form mineralized matrix *in vitro* and *in vivo*.

Keywords

Adipogenic; CD73; CD90; CD146; immunocompromised mice; inflamed periapical tissue; iPAPCs; mesenchymal stem cells; mineralized tissues; osteogenic; STRO-1

Human periapical inflammatory tissues that are formed resulting from endodontic infection are often termed periapical granuloma, and the condition is referred to as apical periodontitis. To form this tissue, periapical bone needs to be resorbed while the inflammation is being developed. After the acute stage of the infection phases out, the chronic stage follows. Histologically, the tissue is characteristically fibrous and infiltrated by inflammatory cells including macrophages, neutrophils, and lymphocytes (1). The tissue contains extensive vascular networks similar to those of the adjacent normal periodontal ligament (2). When the source of infection is absent or removed, the tissue will undergo healing, and new bone will be regenerated. Maeda et al (3) found that periapical lesions contain osteogenic cells that have the potential to differentiate into mature osteoblastic cells and considered that such cells might contribute to osseous healing after root canal treatment. This finding suggests that there is at least the presence of osteogenic progenitors, if not mesenchymal stem cells (MSCs), in the periapical inflamed tissues. Patel et al (4) demonstrated that multipotent MSCs exist in granulation tissue in response to foreign bodies in rats. Our recent studies have shown that stem cell markers, including STRO-1, CD90, CD105, and CD146, can be detected in inflamed pulp tissues by using immunohistochemical analysis. The marker expression is highly associated with vascular structures, and the expression density is higher in inflamed pulp than in normal pulp (5). This could be the result of increased vascularity in inflamed pulp. The question is whether MSCs are present in periapical inflammatory tissues. The purpose of this study, therefore, was to determine whether stem/progenitor cells exist in inflamed periapical lesions.

Materials and Methods

Sample Collection

The patient sample collection in this study conformed to the protocols approved by the Institutional Review Boards of the University of Maryland-Baltimore College of Dental Surgery, Columbia University College of Dental Medicine, and Boston University School of Dental Medicine. The letters of invitation or informed consents of some human subjects reported or described in this article were obtained after the nature of the procedure and possible discomforts and risks had been fully explained. Some samples were obtained with an exempt Institutional Review Board protocol. Inflamed periapical tissues (n = 10) were collected from endodontic apical surgeries in the Department of Endodontics and immediately placed in tissue freezing medium (Triangle Biomedical Sciences, Durham, NC) and kept frozen in -80°C for cryosectioning or placed in culture medium for cell isolation. Dental pulp samples (n = 5) were collected from extracted teeth as described previously (6, 7). For collecting jaw bone samples, freshly extracted teeth were searched for the presence of attached alveolar bone (n = 1). Pieces of the attached bone were carefully removed from the tooth and placed in culture medium for cell isolation. All samples were obtained from generally healthy patients. The inflamed periapical tissues were from patients 27–68 years of age. The extracted teeth were from individuals 16–30 years of age. Cell culture isolation from inflamed periapical tissues followed our previous reports (6–8). In brief, inflamed periapical tissues were minced and digested in a solution of 3 mg/mL of collagenase type I and 4 mg/mL dispase for 20–30 minutes at 37°C . The cells were pelleted and seeded in culture dishes and incubated in α -MEM culture medium with 10% fetal bovine serum, 2 mmol/L glutamine, 100 $\mu\text{mol/L}$ L-ascorbic acid-2-phosphate and antibiotics. On the second day, many cells were attached, and some cells exhibited colony-forming unit-fibroblasts (CFU-Fs). Bone marrow (BM)-derived MSCs (BMMSCs) were obtained from R. S. Tuan (National Institutes of Health, Bethesda, MD) and cultured on the basis of our previous report (6). Dental pulp stem cells (DPSCs) were isolated, as described previously (6), from human dental pulp and cultured by using the above mentioned medium. To isolate cells from alveolar bone, a protocol reported by Song et al (9) was followed. The bone was chipped

into pieces $\sim 1.5 \times 1.5 \times 1.0$ mm in size, and the pieces were placed into culture plates to allow cells to outgrow from the bone fragments. These cells are termed jaw bone-derived MSCs (JBMSCs) and were grown in the same medium as for DPSCs and cells from inflamed periapical tissues.

Antibodies

Primary antibodies used for immunohistochemistry were mouse immunoglobulin G (IgG) control, mouse monoclonal anti-human STRO-1 (Invitrogen Corporation, Carlsbad, CA), mouse monoclonal anti-human CD90 (BD Biosciences, San Jose, CA), and mouse monoclonal anti-MUC18 (CD146) (Invitrogen Corporation). For flow cytometric analysis, purified monoclonal anti-human antibodies to CD34, CD45, and CD105 were purchased from eBioscience (San Diego, CA); purified monoclonal anti-human antibodies to STRO-1 and CD146 were from Invitrogen; and purified monoclonal anti-human antibodies to CD73 were from BioLegend (San Diego, CA). Fluorescein isothiocyanate (FITC) goat anti-mouse IgG/IgM that was used as secondary antibody for flow cytometric analysis was from Invitrogen.

Immunohistochemistry

Frozen tissues were cryosectioned at $8 \mu\text{m}$ and stained for STRO-1, CD 90, and CD146. Sections were fixed with cold acetone at -20°C for 15 minutes, washed in phosphate-buffered saline (PBS), treated with 1.5% hydrogen peroxide for 30 minutes, washed and blocked with 5% normal goat serum (Vector Laboratories, Burlingame, CA) or 2.5% normal horse serum (Vectastain Elite ABC kit; Vector laboratories) for 1 hour. Sections were then incubated with primary antibodies for 1 hour at room temperature. Primary anti-bodies were mixed with 1% bovine serum albumin (BSA) to the following dilutions: STRO-1, 1:25; CD90, 1:10; CD146, 1:50. The sections were then washed, and the appropriate secondary antibodies were added for 1 hour. Secondary antibodies used were biotinylated goat anti-mouse IgM secondary antibody (Vector Laboratories) or IgG secondary antibody. After washing, avidin-peroxidase complex was added and incubated for 30 minutes, followed by washing and the addition of peroxidase substrate solution for 5 minutes. Sections were counterstained with Mayer's hematoxylin solution (Sigma-Aldrich, St Louis, MO) and mounted in aqueous mounting medium (Electron Microscopy Service, Hatfield, PA). For the negative control sections, the nonimmune antibodies were used.

Immunophenotype Analysis

Heterogeneous populations of cells (1×10^5) were stained with purified antibodies against cell surface marker antigens (STRO-1, CD34, CD45, CD73, CD90, CD105, and CD146), followed by incubation with FITC secondary antibodies (BD Biosciences). Subclass-matched antibodies were used as controls. Buffer for staining was made by using 1% BSA in PBS. Flow cytometric analysis was performed with an LSR II Flow Cytometry System (BD Biosystems). Positive cells were defined as those showing a level of fluorescence more than $\sim 99.5\%$ of the corresponding isotype-matched antibodies.

Population Doubling

Cells from inflamed periapical tissues were seeded at low density (200 or 400 cells/well of 12-well plates) and allowed to grow until $\sim 70\%$ – 80% confluence. Cells were then passaged at the same cell density. The population doubling (PD) was calculated at every passage on the basis of our previous report (10). To determine ultimate PDs, cumulative addition of the calculated doubling number from each passage was made until the cells ceased dividing (11). The criterion for cell senescence was that the cells did not divide for 1 month in culture.

Differentiation Induction

Cells were seeded onto 12-well or 48-well plates at 3×10^4 (12-well) or 1×10^4 (48-well) cells/cm² to undergo differentiation stimulation. Subconfluent cultures were incubated for 2–8 weeks in osteogenic/dentinogenic or adipogenic medium (Table 1). At the end of differentiation stimulation, cells were analyzed with either chemical staining (at ~8 weeks) or harvested for RNA isolation (at 2 weeks) for semiquantitative reverse transcription polymerase chain reaction (RT-PCR) to determine the lineage-specific gene expression profile. The primers used for PCR are listed in Table 2. At the end of osteogenic/dentinogenic stimulation, the cultures were fixed with 60% isopropanol for 1 minute, washed with dH₂O, stained with 1% alizarin red S for 3 minutes, and washed with distilled water to assess the formation of the mineralized matrix (7–9). At the end of the adipogenic differentiation, the cells were washed and fixed with 10% formalin and stained with Oil red O as described previously (5). For RT-PCR, total cellular RNA was isolated by using an RNeasy Mini Kit (Qiagen, Valencia, CA) with DNase I (Invitrogen) to remove genomic DNA contaminants. The extracted RNA was reverse transcribed to generate the first strand cDNA by using Superscript III (Invitrogen). The produced cDNA was used as a template for each PCR reaction by using Platinum Blue PCR Supermix (Invitrogen) and the appropriate primers (Table 2). The PCR reaction was performed by using an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with the following thermal cycling steps: 94°C for 2 minutes, denaturing at 94°C for 30 seconds, annealing at 50°C–60°C for 1 minute, extension at 72°C for 1 minute, and 25–30 cycles for the denaturing/annealing/extension. The resulting PCR products were run on 1.5% agarose gel with ethidium bromide (Promega Corp, Madison, WI) stain, and gel images were captured and quantified by using a Gel Doc XR+ w/ Image Lab 3.0 Software (Bio-Rad, Hercules, CA).

In Vivo Transplantation

Cells at passages 1–5 (4×10^6) were mixed with the carrier hydroxyapatite and tri-calcium phosphate (HA/TCP) (40 mg; Berkeley Advanced Biomaterials Inc, Berkeley, CA) and incubated for 90 minutes at 37°C. The mixtures were subcutaneously transplanted into NOD.CB17-Prkdc^{scid}/J (female, 8–12 weeks old) (Jackson Labs, Bar Harbor, ME). Eight weeks after implantation the resected tissues were fixed with 4% paraformaldehyde in PBS (pH 7.2) and decalcified with 10% formic acid in PBS (pH 7.2) for 4 weeks. The samples were dehydrated, embedded in paraffin blocks, and sectioned (6 μ m) for hematoxylin-eosin staining.

Results

Expression of Markers in Inflamed Periapical Tissues

A list of markers has been used to identify the presence of MSCs. We used antibodies against several known MSC markers, STRO-1, CD90, and CD146, to detect the presence of these antigens via an immunohistochemical approach. The results showed that all 3 markers were detected in the inflamed periapical tissues (Fig. 1A–C). Similar to other dental tissues, the staining appears to be associated with vascular structures.

Clonogenic Primary Cultures of Inflamed Periapical Tissue-derived Cells

When cells were isolated and grown in culture dishes, many nonadherent leukocytes were observed floating in cultures at the early stage of cell seeding (Fig. 2A, B). A low number of adherent multinuclear cells, likely osteoclasts, were also noted (not shown). Most adherent cells were fibroblastic with more spindle-shaped morphology and were scattered in cultures. A number of CFU-Fs were observed, and the cells were smaller and more triangular in shape. The ability to form CFU-Fs is one of the important characteristics for MSCs. After

passaging, the CFU-Fs were also observed (Fig. 2C and D); however, the forming ability diminished dramatically. Our PD studies showed that these cells divided very slowly and did not form high-density CFU-F colonies after passaging. The final PDs of those isolated cells (pooled population) from 2 samples were ~23–26. The PD times were 2 days and at later stage up to 10 days.

Cells from Inflamed Periapical Tissues Show Typical MSC Immunophenotype

We performed flow cytometric analysis to detect cell surface marker expression of these isolated cells. As shown in Figure 3A, these cells at passage 2 expressed low levels of STRO-1 and CD146 and high levels of CD73, CD90, and CD105. The expression of CD34 and CD45 was basically negative, indicating the mesenchymal lineage of these cells. Herein we term these cells from inflamed periapical tissues “inflamed periapical progenitor cells (iPAPCs)”. At passage 6, expression levels of STRO-1, CD73, CD90, CD105, and CD146 all decreased (Fig. 3B).

Cells from Inflamed Periapical Tissue Are Highly Osteogenic but Weak in Adipogenic *In Vitro*

After cultured in osteogenic medium for ~8 weeks, iPAPCs showed a large amount of mineralization covering the entire wells revealed by alizarin red stain (Fig. 4A), similar to that produced by other MSCs including BMMSCs, DPSCs, and JBMSCs. When osteogenic gene markers were examined by RT-PCR at 2 weeks, most osteogenic marker genes were either already expressed in the control condition or induced after stimulation including alkaline phosphatase, core-binding factor $\alpha 1$, and osteocalcin. Bone sialoprotein, on the other hand, was not detected or at best minimally induced in an iPAPC sample and the JBMSC sample (Fig. 4B). Various osteogenic genes are expressed or induced by different osteogenic cell types at different time points after induction as reported in the literature (5, 12, 13).

We also determined the adipogenic capacity of iPAPCs. The Oil red O stain was strong in cultures of BMMSCs, showing many enlarged cells containing oil droplets, as shown in Figure 5A. A considerable number of JBMSCs also enlarged, presenting characteristics of adipocytes (Fig. 5B). There were less DPSCs that differentiated into adipocytelike cells, and the size of those cells was much smaller than those in BMMSC cultures (Fig. 5A, C). iPAPC cultures were similar to DPSCs in that very few cells showed adipocyte-like features (Fig. 5D). At best, cells contained very small oil droplets in their cytoplasm. RT-PCR analysis at 2 weeks after induction confirmed the induction of an adipogenic marker lipoprotein lipase (Fig. 5E). iPAPC samples appeared to have already expressed this gene in the control condition.

In Vivo Mineralized Tissue Formation

Tissue regenerative capacity of iPAPCs was determined by using the model of subcutaneous implantation in immunocompromised mice as described in our previous report (8). These cells mixed with HA/TCP were transplanted into the mice for 8 weeks. Histologic examination revealed that iPAPCs formed mineralized tissue deposited against the HA/TCP carrier. DPSCs that are known to form dentin/pulp complexes were used as a positive control. A layer of mineralized tissue was formed around the HA/TCP particles, and the pulp-like tissue was observed adjacent to the mineralized tissue as shown in Figure 6A. iPAPCs also deposited a layer of mineralized tissue generally not as thick as that formed by DPSCs around the HA/TCP. The space between the mineralized tissue was filled by dense fibrous tissues (Fig. 6B–D). No formation of typical bone trabeculae with bone marrow cavities was observed in iPAPC transplants, indicating that unlike the ability of BMMSCs, iPAPCs were not able to form ectopic bone tissues under such experimental settings.

Discussion

Our present studies demonstrated that mesenchymal progenitor cells were present in inflamed periapical tissues as evidenced by the expression of mesenchymal stem cell markers STRO-1 and CD146 in the tissue and in isolated cells in cultures. These iPAPCs showed strong osteogenic potential *in vitro* and were capable of forming mineralized tissues *in vivo*. Periapical inflamed tissues are formed in response to endodontic infection spreading into periapical tissues. The periodontal ligament and bone are destroyed at the affected region and are replaced by inflamed tissues consisting mainly of inflammatory infiltrates and fibroblastic cells supported by the fibrous parenchyma. At certain stages of lesion formation, angiogenesis might be quite active. The mechanisms underlying the development of the apical inflammatory tissues are very complex and have been extensively studied (14–18). However, the kinetics of the healing of the inflamed tissues, ie, regeneration of bone, is less well understood. Most information known is at histologic and cellular level concerning periapical healing and bone regeneration after surgical procedures or root canal treatment (17–22). There has been no study on the possible existence of stem cells in the apical inflamed tissues in terms of their role in regulation of inflammation and healing. Our previous studies showed that stem cells still exist in inflamed pulp tissues, and they are capable of forming pulp/dentin complex *in vivo* (5). The inflamed pulp model might not be the case for periapical inflamed tissue because the nature of the 2 tissues is different. Patel et al (4) demonstrated that multipotent MSCs exist in newly formed granulation tissue in response to foreign bodies in rats. They found that these cells expressed markers of embryonic pluripotent cells (Oct-4 and Nanog) and of adult stem cells (CXCR4 and Thy1.1) as well as produced high levels of vascular endothelial growth factor for up to 10 passages. By fluorescence-activated cell-sorting analysis, these granulation tissue-derived stem cells were positive for stem cell surface markers CD90, CD59, and CD44 and were negative for CD45, which suggests that they were mesenchymal cells and not of hematopoietic lineage. When incubated in specific differentiation medium, these cells transformed into adipogenic, osteogenic, and chondrogenic lineages. However, granulation tissues at later stage appear to lose the potency as MSCs. These granulation tissues were formed in response to a foreign body, a noninfectious material; therefore its nature is also different from the inflammatory periapical tissues. In addition, tissue response in subcutaneous site is likely to be different from that in periapical tissue in terms of the difference in cell types and tissues that are involved. Although STRO-1 and CD146 were detected in inflamed periapical tissues, isolated iPAPCs expressed low levels of STRO-1 and CD146, and their levels further diminished at higher passages. The ability to form CFU-Fs of iPAPCs after passaging was weak, and their PD (~23–26) is relatively lower than those of DPSCs (PD, ~60) and stem cells from apical papilla (PD, ~70) (5, 23). This suggests that their stem cell properties might have been hampered by the presence of inflammation. The DPSCs in inflamed pulp showed lower PD than those from normal pulp, suggesting that inflammation is likely to affect stem cell properties (5). Angiogenesis occurs during the early stage of wound healing when the granulation tissue is formed. The findings by Patel et al suggest that MSCs emerge in the newly formed granulation tissues. They discussed that these MSCs could conceivably come from a local pool of resting stem cells in these tissue sites that expand in response to the inflammatory reaction caused by the presence of a large foreign body. The authors considered it less likely that these MSCs come from circulating MSCs in blood or bone marrow that are mobilized in response to the foreign-body “insult.” This speculation is based on the finding that the contribution of bone marrow cells in a foreign body granulation tissue is minimal. The same consideration might be applied to iPAPCs as well. The newly generated blood vessels expanded from the adjacent periapical tissues might carry new stem cells (eg, pericytes) with them. Our immunostaining data indicated that the tissues express MSC markers, suggesting the presence of stem cells, although their properties might have been altered as discussed above. iPAPCs showed osteogenic potential, especially by the *in*

vitro data. Recently, studies showed that human apical granulation tissues contain osteogenic cells (3). It appears reasonable to speculate that these cells in the inflamed periapical tissues differentiate into osteoblasts and can regenerate periapical bone once the infection source is removed. Further studies are needed to isolate potential MSCs in early stage of inflamed periapical tissue development and to determine their role in periapical bone healing. It has been noted that periapical bone healing begins from the periphery of the lesion and gradually encroaches toward the root apex. If iPAPCs in the inflamed tissues turn into osteoblasts after infection is removed, why does the lesion not heal homogeneously with bone, filling into the previously inflamed space? A study has indirectly indicated that periapical lesions heal faster if the inflamed tissues were surgically removed (24). This implicates that periapical bone healing is slowed down in the presence of inflammation of granulation tissues compared with blood clot-filled apical space after the surgery. Our *in vivo* data demonstrate the inability of iPAPCs to form typical bone structures containing marrow space, suggesting that these cells might be incapable of generating new bone in the lesions, which might explain why the lesion is mainly filled with bone from the periphery, not from within. More investigation is needed to elucidate these phenomena. In addition, the potential role of iPAPCs in the case of pulp revitalization/revascularization should be a subject of future studies.

Acknowledgments

We thank Drs Sophia Lalani and Kristel Tabet (Boston University, Boston, MA) for assisting with the tissue collection.

This work was supported in part by grants from the American Association of Endodontists Foundation and National Institutes of Health R01 DE019156 (G.T.-J.H.).

References

1. Metzger, Z.; Abramovitz, I. Ingle's endodontics. 6th ed. B C Decker Inc; Hamilton, Ontario: 2008. Periapical lesions of endodontic origin. chapter 13
2. Davey KJ, Perrier S, Ohe G, et al. Assessment of vascularity as an index of angiogenesis in periradicular granulomas: comparison with oral carcinomas and normal tissue counterparts. *Int Endod J.* 2008; 41:987–96. [PubMed: 19133088]
3. Maeda H, Wada N, Nakamuta H, Akamine A. Human periapical granulation tissue contains osteogenic cells. *Cell Tissue Res.* 2004; 315:203–8. [PubMed: 14648194]
4. Patel J, Gudehithlu KP, Dunea G, Arruda JA, Singh AK. Foreign body-induced granulation tissue is a source of adult stem cells. *Transl Res.* 2010; 155:191–9. [PubMed: 20303468]
5. Alongi DJ, Yamaza T, Song Y, et al. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. *Regen Med.* 2010; 5:617–31. [PubMed: 20465527]
6. Huang GT, Yamaza T, Shea LD, et al. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an *in vivo* model. *Tissue Eng Part A.* 2010; 16:605–15. [PubMed: 19737072]
7. Huang GTJ, Sonoyama W, Chen J, Park S. In vitro characterization of human dental pulp cells: various isolation methods and culturing environments. *Cell Tissue Res.* 2006; 324:225–36. [PubMed: 16440193]
8. Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci.* 2000; 97:13625–30. [PubMed: 11087820]
9. Song L, Young NJ, Webb NE, Tuan RS. Origin and characterization of multipotential mesenchymal stem cells derived from adult human trabecular bone. *Stem Cells Dev.* 2005; 14:712–21. [PubMed: 16433626]
10. Huang GTJ, Shagaramanova K, Chan SW. Formation of odontoblast-like cells from cultured human dental pulp cells on dentin *in vitro*. *J Endod.* 2006; 32:1066–73. [PubMed: 17055908]

11. Blazer S, Khankin E, Segev Y, et al. High glucose-induced replicative senescence: point of no return and effect of telomerase. *Biochem Biophys Res Commun.* 2002; 296:93–101. [PubMed: 12147232]
12. Liu J, Jin T, Ritchie HH, Smith AJ, Clarkson BH. In vitro differentiation and mineralization of human dental pulp cells induced by dentin extract. *In Vitro Cell Dev Biol Anim.* 2005; 41:232–8. [PubMed: 16223338]
13. Viereck V, Siggelkow H, Tauber S, Raddatz D, Schutze N, Hufner M. Differential regulation of Cbfa1/Runx2 and osteocalcin gene expression by vitamin-D3, dexamethasone, and local growth factors in primary human osteoblasts. *J Cell Biochem.* 2002; 86:348–56. [PubMed: 12112004]
14. Stashenko P. Role of immune cytokines in the pathogenesis of periapical lesions. *Endod Dent Traumatol.* 1990; 6:89–96. [PubMed: 2079017]
15. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. *Crit Rev Oral Biol Med.* 1998; 9:498–521. [PubMed: 9825224]
16. Nair PNR. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med.* 2004; 15:348–81. [PubMed: 15574679]
17. Fouad AF, Walton RE, Rittman BR. Induced periapical lesions in ferret canines: histologic and radiographic evaluation. *Endod Dent Traumatol.* 1992; 8:56–62. [PubMed: 1521506]
18. Fouad AF, Walton RE, Rittman BR. Healing of induced periapical lesions in ferret canines. *J Endod.* 1993; 19:123–9. [PubMed: 8509750]
19. Asgary S, Eghbal MJ, Ehsani S. Periradicular regeneration after endodontic surgery with calcium-enriched mixture cement in dogs. *J Endod.* 2010; 36:837–41. [PubMed: 20416429]
20. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery: 2—the dissectional wound. *J Endod.* 1991; 17:544–52. [PubMed: 1812204]
21. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery: I—the incisional wound. *J Endod.* 1991; 17:425–35. [PubMed: 1811035]
22. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery: III—the osseous excisional wound. *J Endod.* 1992; 18:76–81. [PubMed: 19186423]
23. Sonoyama W, Liu Y, Fang D, et al. Mesenchymal stem cell-mediated functional tooth regeneration in Swine. *PLoS ONE.* 2006; 1:e79. [PubMed: 17183711]
24. Kvist T, Reit C. Results of endodontic retreatment: a randomized clinical study comparing surgical and nonsurgical procedures. *J Endod.* 1999; 25:814–7. [PubMed: 10726528]

Figure 1. Immunohistochemical staining of inflamed periapical tissues. Representative data from 1 sample indicate positive staining of STRO-1 (A), CD90 (B), and CD146 (C) (arrowheads). (D) Nonimmune control. Scale bar: 100 μ m (A–D).

Figure 2. Growth of cells isolated from inflamed periapical tissues. (*A* and *B*) Cells released from the granulation tissue at passage 0. Many floating cells shown in (*A*) are the leukocytes infiltrated in the inflamed tissue. Colony formation (CFU-F) is shown in (*B*). Colony formation reappeared at passage 1 (*C* and *D*). Scale bar: 500 μm (*A–D*).

Figure 3. Immunophenotyping of cells from inflamed periapical tissues. Heterogeneous pooled population of cells at passages 2 (*A*) and 6 (*B*) were analyzed with flow cytometry to detect cell surface marker expression. Dashed peaks are the controls, and solid peaks are the specific antibody staining.

Figure 4.

Osteogenic differentiation of iPAPCs and other MSCs. (A) Alizarin red stain of mineral deposits in cultures. BMMSCs (20, sample from a 20-year-old man; 63, from a 63-year-old woman), iPAPCs (numbers represent different samples of inflamed periapical tissues; #5b is a subclone, the rest are pooled cells; #1, 60-year-old man; #2, 41-year-old man; #5, 27-year-old woman), DPSCs (~20-year-old donor), JBMSCs (21-year-old woman). All cells at passages 2–3 had undergone osteogenic stimulation for 7.5 weeks. Cont, control; Osteo, osteogenic stimulation. (B) RT-PCR analysis of osteogenic markers of cells harvested after 2-week stimulation. Cells used were the same as those in (A). ALP, alkaline phosphatase; BSP, bone sialoprotein; C, control; CBFA1, core-binding factor $\alpha 1$; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; O, osteogenic stimulation; OCN, osteocalcin.

Figure 5.

Adipogenic differentiation of iPAPCs and other MSCs. All cells at passages 2–3 had undergone adipogenic stimulation for 8 weeks and were stained with Oil red. (A) BMMSCs (sample from 20-year-old man); (B) JBMSCs (21-year-old woman); (C) DPSCs; (D) iPAPCs (sample #1, 60-year-old man; sample #2, 41-year-old man). Scale bar: 50 μ m (A–D). (E) RT-PCR analysis of adipogenic marker of cells harvested after 2-week stimulation. A, adipogenic stimulation; C, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPL, lipoprotein lipase.

Figure 6.

In vivo formation of mineralized tissues. Cells mixed with HA/TCP were subcutaneously transplanted into SCID mice. After 8 weeks, the resected tissues were processed for hematoxylin-eosin staining. (A) DPSCs at passage 3 as positive controls. (B) iPAPCs, a subclone at passage 4, sample #5, 27-year-old woman. (C) iPAPCs, pooled cells at passage 1, sample #1, 60-year-old man. (D) iPAPCs, a subclone at passage 5, sample #2, 41-year-old man. ct, fibrous connective tissues; HA, HA/TCP; p, dental pulp-like. *Arrowheads* indicate mineralized tissue. Scale bar: 100 μ m (A–D).

\$watermark-text

\$watermark-text

\$watermark-text

TABLE 1

Lineage-specific Differentiation Inducing Media

Lineage	Medium	Serum	Supplementation
Osteogenic/dentinogenic	DMEM	10% FBS	10 nmol/L dexamethasone, 10 mmol/L β -glycerolphosphate, 50 μ g/mL ascorbate phosphate, and 10 nmol/L 1, 25 dihydroxyvitamin D3
Adipogenic	DMEM	10% FBS	1 μ mol/L dexamethasone, 1 μ g/mL insulin, and 0.5 mmol/L 3-isobutyl-1-methylxanthine (IBMX)

Medium/serum and supplement were changed every 3 days.
FBS, fetal bovine serum.

\$watermark-text

\$watermark-text

\$watermark-text

TABLE 2

Primers Used for RT-PCR

Lineage	Gene	Primer (5'–3') Sense Antisense	Product size (base pairs)
Osteogenic/dentinogenic	ALP	CCACGTCTTCACATTTGGTG	196
		AGACTGCGCCTGGTAGTTGT	
	BSP	AAAGTGAGAACGGGAACCT	161
		GATGCAAAAGCCAGAAATGGAT	
Adipogenic	CBFA1	TTTGCACTGGGTCATGTGTT	156
		TGGCTGCATTGAAAAGACTG	
		GGCAGGAGGTAGTGAAGAG	230
Housekeeping	GAPDH	CTGGAGAGGAGCAGAACTGG	
		AGTGGCCAAATAGCACATCC	186
		CCGAAAGATCCAGAAATTCCA	194
		CAAGGCTGAGAACGGGAAGC	
		AGGGGCAGAGATGATGACC	

ALP, alkaline phosphatase; BSP, bone sialoprotein; CBFA1, core-binding factor al; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPL, lipoprotein lipase; OCN, osteocalcin.