Putative Stem Cells in Human Dental Pulp with Irreversible Pulpitis: An Exploratory Study

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Abstract

Introduction: Although human dental pulp stem cells isolated from healthy teeth have been extensively characterized, it is unknown whether stem cells also exist in clinically compromised teeth with irreversible pulpitis. Here we explored whether cells retrieved from clinically compromised dental pulp have stem cell–like properties. **Methods:** Pulp cells were isolated from healthy teeth (control group) and from teeth with clinically diagnosed irreversible pulpitis (diseased group). Cell proliferation, stem cell marker STRO-1 expression, and cell odonto-osteogenic differentiation competence were compared. **Results:** Cells from the diseased group demonstrated decreased colony formation capacity and a slightly decreased cell proliferation rate, but they had similar STRO-1 expression and exhibited a similar percentage of positive *ex vivo* osteogenic induction and dentin sialophosphoprotein expression from STRO-1–enriched pulp cells. **Conclusions:** Our study provides preliminary evidence that clinically compromised dental pulp might contain putative cells with certain stem cell properties. Further characterization of these cells will provide insight regarding whether they could serve as a source of endogenous multipotent cells in tissue regeneration–based dental pulp therapy. *(J Endod 2010;36:820–825)*

Key Words

Dental pulp, irreversible pulpitis, stem cell

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Pulpitis-associated pain contributes to a substantial financial and societal burden and affects quality of life. In addition to pain and suffering, pulp infection can also cause severe and at times even fatal systemic infection (1). Appropriate management of dental pulp infection and inflammation has tremendous significance in maintaining general health. Although immediate treatment is indispensable for a better prognosis, current endodontic therapies typically lead to destroying or compromising pulp vitality. In adult teeth, even when the lesion affects only part of the pulp, the entire pulp tissue is often removed (root canal therapy). The resulting nonvital teeth often require extensive restorations, which in turn increase the cost and the risk of complications. In primary teeth, pulpotomy treatment involves application of formaldehyde, a toxic, mutagenic chemical that can be absorbed into the body (2, 3). Therefore, it is important to develop biocompatible treatments directed at maintaining pulp vitality and increasing tooth longevity.

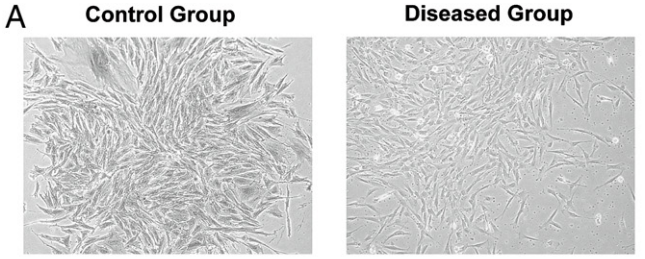
The discovery of dental pulp stem cells has important implications in both medical and dental fields. Postnatal dental pulp stem cells (DPSCs) (4, 5), stem cells from exfoliated deciduous teeth (SHED) (6), and stem cells from the apical papilla (SCAP) (7, 8) have stem cell properties including multipotent and self-renewal capacities. They are able to differentiate into a variety of cell types including odontoblasts *in vitro* (4, 9) and form dentin/pulp-like complexes *in vivo* (5, 10). DPSCs and SCAP also have self-renewal capacity evidenced by animal studies with human cell transplants (9, 11); particularly, human SCAP and DPSCs can regenerate a pulp-like structure *de novo* with established vascularity and dentin formation when transplanted in a tooth fragment carrier (11). The discovery of DPSCs and SCAP makes it possible to develop a biocompatible treatment based on endogenous pulp repair or regeneration.

However, when a dental pulp is diagnosed with irreversible pulpitis, the available pulp tissue is believed to be inflamed or infected. In this regard, it is unknown whether these damaged dental pulps still contain stem cells with competent proliferation and differentiation capacities. Answering these questions is critical for the development of autologous stem cell–based pulp therapy strategies to achieve *in situ* regeneration with optimal growth factors and matrix. In this exploratory study, we hypothesized that the pulp cells residing in pulp clinically diagnosed with irreversible pulpitis might still have stem cell potential similar to healthy pulp cells and therefore might be a resource for autologous pulp regeneration.

Materials and Methods

Subjects

Pulp tissues were obtained from permanent teeth of patients (6–40 years of age) recruited from outpatient clinics of the University of North Carolina at Chapel Hill (UNC-CH) School of Dentistry under a protocol approved through the Institutional Research Board committee of UNC-CH after consent. Healthy pulp tissues were collected from 8 patients undergoing orthodontic molar extraction (control group). All teeth were free of carious lesions. Compromised dental pulps were obtained from 8 patients with irreversible pulpitis (diseased group) that required treatment procedures to remove pulp tissue from involved teeth. The diagnosis of irreversible pulpitis was determined by endodontic specialists on the basis of clinical assessment, including history of



	Control Group	Diseased Group
% of cell grow	100	87.5
% of single cell derived colony formation	100	75

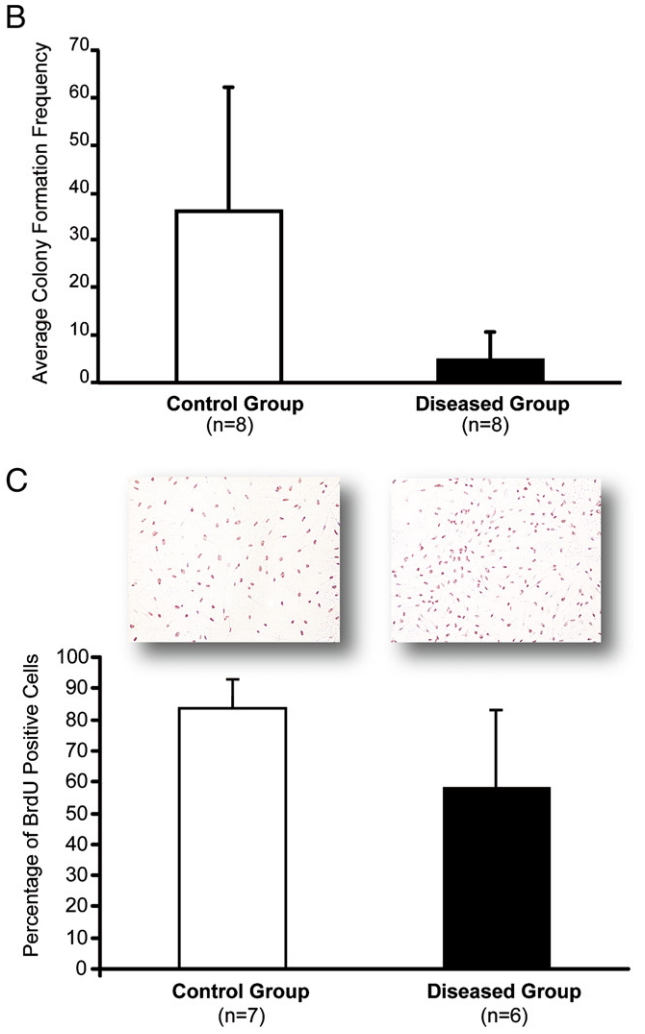


Figure 1. Single cell–derived colony formation in primary culture and cell proliferation in expanded cultures from both control and diseased groups. (*A*) Colony morphology is similar in control and diseased groups. Pictures show part of a colony, taken between days 10–14 (objective, 10×). (*B*)Colony formation frequency was defined as the number of units with more than 50 cells. Cultures were fixed with 10% buffered formalin phosphate between days 10–21 and then stained with 0.1% crystal violet. Each experiment was performed in triplicates. Wilcoxon rank sum test, $P = .021$; ratio of means (ie, fold change) 90% confidence interval (3.1–18.8). (*C*) Average percentage of BrdU-positive cells in each group was calculated. BrdU was incorporated into proliferating cells and then detected by a monoclonal anti-BrdU. Percentage of positive cells of each sample was recorded by counting 5 randomized fields (objective, 10× or 20×), and the mean percentage of positive cells was compared. Pictures are representative examples from each group taken at 10×. Error bars represent standard deviation. Wilcoxon rank sum test, $P = .036$; fold change 90% confidence interval (1.04–2.01).

spontaneous pain and intense, lingering pain to cold stimulus. The vitality of the pulp was confirmed on access. Teeth with completely necrotized pulp tissue were excluded.

Cell Culture

Healthy dental pulp tissue was harvested as previously described (5, 12). Extracted healthy teeth were sterilized with iodine and scaled thoroughly to remove all periodontal and periapical tissue before drilled and sectioned in half to obtain pulp tissue. Diseased pulp tissues were collected from pulp chambers with a sterile broach after complete exposure of pulp chamber and transferred into sterile α-minimum essential medium with 2 mmol/L L-glutamine (α-MEM; Gibco, Gaithersburg, MD), penicillin, and streptomycin. All pulp tissues were washed with α-MEM with 10% fetal bovine serum (FBS; Gibco), digested, and cultured as described (5, 12). Antibiotics (penicillin and streptomycin) were used in all washing, digesting buffer, and culture media to minimize bacteria contamination. Primary cells were passed to second passage (named passage 1, P1), a portion of which was cryopreserved for later expansion. P2–P5 cells were used in most *in vitro* assays. Each control and diseased pulp tissue was processed, cultured, and evaluated separately in all experiments.

Single Cell–derived Colony Formation Assay

To assess single cell–derived colony formation efficiency, primary cells were seeded into 6-well plates at a live cell concentration of 0.5×10^5/mL as described above. Single cell–derived colonies were defined as those units with more than 50 cells. The number of colonies was counted on the day before colonies would merge together, usually between days 13–16. For those samples with limited colonies, the number was counted as late as 21 days of culture.

Bromodeoxyuridine Cell Proliferation Rate Assay

Cells from each sample were seeded onto 3 coverslips in 6-well plates and cultured until fully attached. Then the cells were starved in serum-free medium for 24 hours before adding back normal medium with bromodeoxyuridine (BrdU; Roche, Indianapolis, IN). Proliferation was evaluated after 24 hours according to the Zymed BrdU attaining kit instructions (Invitrogen, Carlsbad, CA). Briefly, BrdU incorporation was detected by a biotinylated monoclonal anti-BrdU antibody. Staining was visualized after adding streptavidin-peroxidase and diaminobenzidine (DAB). Positive cell percentage in each coverslip was recorded by counting cells in 5 randomly selected fields under a light microscope (Nikon ECLIPS TS100; Tokyo, Japan). The average BrdU-positive cell percentage of each sample was calculated, and mean values from each group were compared.

STRO-1 Expression Evaluation and STRO-1–Positive Cell Purification

STRO-1 has been used as a stromal stem cell surface marker in characterizing stem cell composition in mesenchymal stem cells (13). To investigate whether diseased dental pulp still contains presumptive progenitor cells, we evaluated STRO-1 expression on expanded cells by flow cytometry. Cells from each patient sample were harvested at 70% confluence, washed, blocked in 10% FBS on