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Application of eGFP to label human periodontal ligament stem cells in periodontal tissue engineering

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ABSTRACT

Objectives: To establish human periodontal ligament stem cells (hPDLSC) with high and stable expression of enhanced green fluorescent protein (eGFP) and to obtain an ideal vector expression system that suitable for gene therapy in periodontal tissue engineering.

Methods: hPDLSCs were transfected with eGFP for 48 h via different MOI (25, 50, 100, 200 and 400) by lentiviral vector, the transfection efficiency was evaluated by fluorescent microscopy and flow cytometry, and transfected hPDLSCs proliferation was evaluated by MTT. Pluripotent, differentiation capacity and ALP expression status were determined further. Osteoblast-associated genes expressions for osteogenesis were evaluated by quantitative-PCR. In addition, rat molar periodontal fenestration defect model was used for evaluating periodontal tissue engineering.

Results: The transfection efficiency after 48 h were 44.7%, 60.9%, 71.7%, 85.8%, and 86.9% respectively. There was no significant effect of transfection (at different MOI levels of 25, 50, 100, and 200) on the proliferation of hPDLSCs (designated as eGFP-hPDLSCs) compared with hPDLSCs ($P > 0.05$). However, proliferation of eGFP hPDLSCs at MOI 400 became slower ($P < 0.05$). Both eGFP hPDLSCs and hPDLSCs were able to differentiate into osteocytes and adipocytes under certain conditioned media. At 7 days, expression levels of *COL-1*, *RUNX2* in hPDLSCS were higher than those in eGFP hPDLSCs ($P < 0.05$); expression levels of *ALP* and *OPN* in eGFP hPDLSCs were similar to those in hPDLSCs ($P > 0.05$). Newly regenerated bone formation was observed in the defect model used.

Conclusions: Among the transfection conditions, 48 h transfection at MOI 200 is optimal for labelling hPDLSCs with eGFP in a lentiviral vector. There is no change in capability of the eGFP hPDLSCs osteogenesis. The lentiviral vector with eGFP is an appropriate expression vector system and hPDLSCs are ideal seeding cells for gene therapy in periodontal tissue engineering.

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1. Introduction

Periodontitis is a chronic inflammation occurring in deep periodontal tissues[1] and can cause loss of tooth-supporting periodontal tissues, such as bone, cementum and periodontal ligament (PDL).[2] The ultimate goal of periodontal treatment is to regenerate lost periodontal tissues. Stem cell-based therapeutic approaches for bone regeneration have been used in clinical trials.[2] Identification of a reliable cell source is a key step in tissue engineering for periodontal restoration. Periodontal ligament stem cells (PDLSCs) are an undifferentiated stem cell population presented in the periodontal ligament and have abilities of self-renewal and differentiation potential.[3] PDLSCs play an important role in reconstruction of orthodontic tooth movement[4,5] and become a new research area in dental implantation,[6,7] which aims to reconstruct normal periodontal structure and mimic nature tooth environment, thus it may become the most direct seeding cells in periodontal tissue regeneration. Previous studies have showed that PDLSCs mainly localized on paravascular region of periodontal issues and were able to migrate from alveolar bone into periodontal ligament region and differentiate into cementoblasts.[8–10] Studies have shown that PDLSCs possess mesenchymal characteristics, such as colony formation ability, high proliferation rate, expression of mesenchymal stem cell-specific surface markers, differentiation capabilities into osteoblasts, adipocytes, chondrocytes and neural cells in vitro.[11,12] For periodontal regeneration, PDLSCs was thought to be one of the most promising cell types in cell-based regeneration. PDLSCs in new cementum and periodontal ligament-like tissues was superior to bone marrow stromal cells and periosteal cells,[13] and also become an ideal seed cells carrier for gene therapy in periodontal regeneration.

Gene therapy can be broadly defined as treatment of a disease or medical disorder by introduction of therapeutic genes into appropriate cellular targets. These therapeutic genes can correct deleterious consequences of mutations in specific genes or restore cell functions to overcome various disease disorders.[14] For a successful gene therapy, exogenous therapeutic genes have to be specifically, efficiently, and stably incorporated into target cells. So, efficient gene delivery and appropriate expression vector systems are key factors to achieve such purposes and directly affect their choice of treatment efficiency and safety. Lentiviral vectors have been widely used in fundamental biological research, functional genomics, and gene therapy.[15] Some studies have shown that lentiviral vectors can be used in bone marrow, breast and other tissue cells to achieve effective and sustained transgene expression.[16,17] However, use of PDLSCs as lentiviral vectors' target cells, an appropriate expression vector system and an ideal seeding cell for successful gene therapy in periodontal tissue regeneration have been less studied. The current study focused on labelling human PDLSCs by eGFP in lentiviral vector, and evaluated biological characteristics of the transfected cells, and provided sufficient and suitable cells for effective gene therapy in periodontal tissue regeneration.

2. Materials and methods

2.1. Isolation and culture of periodontal ligament stem cells

hPDLSCs were isolated and cultured according to the previously describe.[3] Briefly, 24 human premolars extracted from 16 healthy voluntary donors that between16 and 18 years of age were used under the approved guidelines set by Ethics Committee of Shandong University. PDL tissues were separated from root surface and were minced into pieces of small size. The minced tissues were incubated with 3 mg/ml collagenase type I (Sigma) and 4 mg/ml dispase (Sigma) in α-MEM (Gibco) at 37 °C 1 h. Single cells in suspension were obtained by passing through a strainer (pore size: 70 mm from BD Falcon Labware). Then the cells were seeded in 10 cm petri dishes containing α-MEM supplemented with 15% FBS (Gibco), 100 mM L-ascorbic acid 2-phosphate (Sigma), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco), and incubated at 37 °C in 5% CO_2. Cells at passages P3–P5 were used for following experiments.

2.2. Colony-forming unit (CFU) assay

CFU assay was performed to determine putative MSCs (mesenchymal stem cells) from periodontal ligaments. Cells were plated at 100 cells/ml onto 10 cm petri dishes. After 14 days, the cells were fixed by 4% formaldehyde and then stained with crystal violet (Sigma).

2.3. Flow cytometric analysis

Approximately 5×10^5 hPDLSCs were incubated with specific phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibodies against human CD29, CD105, HLA-DR and SSEA-4 (Biolegend); CD34 (eBioscience); CD44 and CD90 (R&D). Flow cytometry data were analyzed using CellQuest (BD Biosciences) analysis software.

2.4. eGFP genes transfection

Lentiviral vector with enhanced green fluorescent protein (pBPLV-eGFP, a gift from Professor Luigi Naldiai) was used to directly trace distribution and differentiation of MSCs in vivo. Lentiviral eGFP expression vector was firstly transfected into the packaging cells 293FT, then high-level lentiviruses particles packed in culture supernatant were collected round 48–72 h. 2×10^5 MSCs were inoculated in a 25 cm^2 culture flask 24 h with 1:1 mix of viral supernatant:growth medium with 5 μg/ml polybrene (Sigma). Then the eGFP-positive cells were chosen by Fluorescence Activated Cell Sorting Analysis. These cells were applied to following research experiments.

2.5. Rat molar periodontal fenestration defect model

A rat periodontal defect model was modified from King et al.[18]. Six SD rats were used. Alveolar bones over the mandibular first molar roots were separated using the dental drill (GX-35EM, NSK Nakanishi Inc., 1 mm diameter). Periodontal window