A Comparative Evaluation of Concentrated Growth Factor and Platelet-rich Fibrin on the Proliferation, Migration, and Differentiation of Human Stem Cells of the Apical Papilla

Shebin Hong, MD, Weiting Chen, MD, and Beizhan Jiang, PhD

Abstract

Introduction: Concentrated growth factor (CGF) is considered to be a natural biomaterial that is better than platelet-rich fibrin (PRF) in bone regeneration, but there is little information acquired in regenerative endodontics. Therefore, the purpose of this study was to evaluate their effects on the proliferation, migration, and differentiation of human stem cells of the apical papilla (SCAPs). **Methods:** CGF- and PRF-conditioned medium were prepared using the freeze-dried method. SCAPs were isolated and identified. The proliferative potential of SCAPs was investigated using the Cell Counting Kit-8 (KeyGen Biotech, Nanjing, China). The migration capacity was analyzed using transwell assays, and the mineralization ability was determined by alizarin red S staining. The expression levels of alkaline phosphatase, bone sialoprotein, dentin matrix protein 1, and dentin sialophosphoprotein were determined by quantitative polymerase chain reaction. **Results:** The cultured cells exhibited mesenchymal stem cell characteristics. The growth rate and migratory cell numbers of the CGF and PRF groups were significantly greater than those of the control group. The mineralized areas in the CGF and PRF groups were significantly larger than those in the control group after incubation for 7 days and 14 days. The expression levels of osteogenic/odontoblast-related genes were reduced on day 7, but they were dramatically enhanced on day 14, and the related gene expression levels in the PRF group were higher than those in the CGF group. **Conclusions:** Both CGF and PRF can promote the proliferation, migration, and differentiation of SCAPs. CGF may be a promising alternative in regenerative endodontics. *(J Endod 2018;■:1–7)*

Key Words

Concentrated growth factor, platelet-rich fibrin, regenerative endodontics, stem cells of the apical papilla

Significance

This article has extended the data on the potential application of CGF and PRF in regenerative endodontics and gives great instructions on the treatment of immature teeth with pulp necrosis and periapical periodontitis.

Pulpitis and periapical periodontitis are the most common diseases in dental clinical treatment, and root canal therapy is the most effective method of treatment. However, many defects still may exist, including root fractures, perforation, and instrument separation. Hence, regenerative endodontics, which attempts to conserve vital pulp and promote pulp/dentin revitalization, has drawn more attention over the past decades (1) and has even been suggested to be an alternative treatment for young permanent teeth with pulp necrosis (2).

In recent years, regenerative endodontics has presented new possibilities for the treatment of necrotic immature permanent teeth based on the meticulous combination and interplay of 3 key elements for tissue regeneration, namely stem cells, bioactive molecules, and scaffolds (3). Stem cells of the apical papilla (SCAPs), which are derived from an embryoniclike soft tissue located at the apex of the incompletely developed root, were first isolated and characterized by Sonoyama et al (4). Compared with dental pulp stem cells, SCAPs exhibit a more pronounced population doubling capacity, an enhanced proliferation rate, and mineralization potential, indicating a more potent stem cell population (4, 5). Given their origin from developing tissue, SCAPs may be a better stem cell source for pulp tissue engineering (5, 6).

Platelet concentrates, which are known as bioscaffolds and a reservoir of cytokines, have been used for tissue regeneration in current clinical medicine (7). Platelet-rich plasma, the first generation of platelet concentrates, has been shown to enhance wound healing (8, 9). However, there is great debate on its biosecurity and stability because of the artificial thrombin and anticoagulant additive (10, 11). Platelet-rich fibrin (PRF), the second generation of platelet concentrates, has drawn more attention because of its simple production process and natural component without any artificial additives. As a reservoir of abundant cytokines, it can promote

From the Department of Operative Dentistry and Endodontics, School and Hospital of Stomatology, Tongji University, Shanghai Engineering Research Center of Tooth Restoration and Regeneration, Shanghai, China.

Address requests for reprints to Dr Beizhan Jiang, Department of Operative Dentistry and Endodontics, School and Hospital of Stomatology, Tongji University, Shanghai Engineering Research Center of Tooth Restoration and Regeneration, No. 399, Middle Yan Chang Road, Shanghai 200072, China. E-mail address: jiangbeizhan@tongji.edu.cn

0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2018.03.006

cell recruitment, proliferation, and differentiation (12–14). In recent years, the effects of PRF on pulp regeneration have been proven by animal models and clinical studies (15–17). Concentrated growth factor (CGF) is the latest generation of platelet concentrate. CGF appears to contain more abundant cytokines and was substantially studied in bone regeneration (14, 18), but there has been little research performed in regenerative endodontics. Therefore, the purpose of this study was to evaluate its effects on the proliferation, migration, and differentiation of SCAPs.

Materials and Methods

Isolation and Characterization of SCAPs

The study protocol was approved by the Institutional Review Board of the Affiliated Stomatology Hospital of Tongji University, Shanghai, China, and ethics committee approval was obtained.

Normal impacted third mandibular molars without apical closure were collected from 3 healthy patients (14–20 years old) who gave informed consent in the dental clinic at the Affiliated Stomatology Hospital of Tongji University. The SCAPs were isolated and cultured according to a previously described method (4). Briefly, apical papillae were removed from the root apex and were minced into pieces. The enzyme digestion method was subsequently performed to treat the minced tissues. The separated cells were cultured in Dulbecco modified Eagle medium (DMEM; Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD), 100 U/mL penicillin G, and streptomycin (Sigma-Aldrich, St Louis, MO). The SCAPs were subcultured from the third generation to the fifth generation, and these cells were used in this study.

The origin of the cells was determined by immunocytofluorescent staining using vimentin and cytokeratin 19 antibodies (Boster, Wuhan, China). The cells were counterstained with 4',6-diamidino-2-phenylindole to visualize the nuclei and were observed under a fluorescence microscope. The characterization of SCAPs was analyzed by flow cytometry and multilineage differentiation assays as previously described (19). In brief, the CD45, CD90, and CD146 cell markers were investigated by the flow cytometry test kit (R&D Systems, Minneapolis, MN). The isotype served as the negative control. For the multilineage differentiation assay, alizarin red S staining and oil red O staining were used to identify the mineralized nodules and lipid droplets after the cells were incubated with different inducing medium for 3 weeks.

Conditioned Medium Preparation

Venous blood (10 mL) was collected from each participant after providing informed consent. Immediately, CGF and PRF were fabricated according to a previously reported protocol (20, 21). Conditioned medium (CM) was prepared as described previously with slight modifications (22). In brief, the isolated CGF and PRF membranes were frozen overnight in a vacuum freeze dryer. To harvest the cytokines, the lyophilized membrane was pulverized and immersed in 10 mL DMEM. The medium was collected after incubation at 37°C for 24 hours and was centrifuged to remove the red blood cells. CM was completed after being supplemented with 10% fetal bovine serum and 1% antibiotic. Three concentrations of 1 CGF or 1 PRF (1 CGF or PRF membrane dissolved in 10 mL DMEM), 1/2 CGF or 1/2 PRF, or 1/4 CGF or 1/4 PRF were used.

Cell Proliferation Assay

The SCAPs were seeded on a 96-well plate (Corning Inc, Corning, NY) at a density of 2 × 103 cells per well and were cultured with different concentrations of CM for 7 days. Normal medium was used as the control group. All media were changed every 2 days. A cell counting kit (Keygen, Nanjing, China) was used to analyze the cell numbers. The optical density values were measured using a microplate reader (Bio-Tek, Hercules, CA) at 450 nm. The results from different groups were compared. After analyzing the results comprehensively and for the sake of research convenience, we selected the concentration of 1/2 CGF or 1/2 PRF for this study.

Cell Migration Assay

Twenty-four plates of Transwell Filter Inserts (Corning Inc) were used to investigate the migratory capacity of SCAPs after being treated with CGF and PRF for 12 and 24 hours. Normal media with or without serum served as the positive and negative control groups, respectively. The migrated cells were stained with crystal violet and were counted randomly in 6 microscope fields. The cell numbers per field were calculated using ImageJ software (version 10.2; National Institutes of Health, Bethesda, MD), and the average was analyzed using GraphPad Prism v4.0 software (GraphPad Software, La Jolla, CA).

Mineralization Induction

Alizarin red S staining was performed to detect the mineralization nodules of the SCAPs after culture in CGF and PRF for 7 and 14 days. In brief, the paraformaldehyde-fixed cells were stained with 2% alizarin red S solution (Sigma-Aldrich, St Louis, MO) and were photographed after several steps of washing.

Real-time Quantitative Polymerase Chain Reaction

The messenger RNA expression levels of alkaline phosphatase (ALP), bone sialoprotein (BSP), dentin matrix protein 1 (DMP-1), and dentin sialophosphoprotein (DSPP) were determined by quantitative polymerase chain reaction. Briefly, the SCAPs treated with different medium were lysed by Trizol reagent (Life Technologies, Carlsbad, CA), and total RNA was isolated. The extracted RNA was reverse transcribed using a complementary DNA synthesis kit (Roche, Schlieren, Switzerland), and the relative messenger RNA expression of the target gene was analyzed using the FastStart Essential DNA Green Master (Roche). The primer sequences for ALP, BSP, DSPP, and DMP-1 (Sango Biotech, Shanghai, China) are listed in Table 1.

TABLE 1. The Primer Sequence Used for Quantitative Real-time Polymerase Chain Reaction Analysis

Gene	Forward primer sequence	Reverse primer sequence
ALP	GTGAACCGCAACTGGTACTC	GAGCTGCGTAGCGATGTCC
BSP	CACTGGAGCCAATGCAGAAGA	TGGTGGGGTTGTAGGTTCAAA
DMP-1	CTCCGAGTTGGACGATGAGG	TCATGCCTGCACTGTTCATTC
DSPP	TTTGGGCAGTAGCATGGGC	CCATCTTGGGTATTCTCTTGCCT

ALP, alkaline phosphatase; BSP, bone sialoprotein; DMP-1, dentin matrix protein 1; DSPP, dentin sialophosphoprotein.