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ORIGINAL ARTICLE

The effects of calcium silicate cement/fibroblast growth factor-2 composite on osteogenesis accelerator in human dental pulp cells

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Abstract *Background/purpose:* To examine the effects of fibroblast growth factor-2 (FGF-2)/calcium silicate (CS) cement on material characters and *in vitro* primary human dental pulp cell (hDPC) behavior.
Materials and methods: Setting time and diametral tensile strength (DTS) of CS and CS/FGF-2 composite were measured. PrestoBlue assay was used for evaluating primary hDPC proliferation. Alkaline phosphatase and osteocalcin expression in HDPCs cultured on the specimens were determined by enzyme-linked immunosorbent assay. One-way analysis of variance was used to evaluate the significance of the differences between the mean values.
Results: Setting time and DTS of CS were not significantly different ($P > 0.05$) between CS hydration with H_2O or FGF-2. Cell proliferation and osteogenic properties increased significantly ($P < 0.05$) with FGF-2 mixed CS. The CS/FGF-2 composite enhanced hDPC proliferation and osteogenic differentiation as compared to pure CS cement.
Conclusion: CS combined with FGF-2 is biocompatible with hDPCs. It not only promotes hDPC proliferation but also helps in differentiating reparative hard tissue. Thus, we suggest that the CS/FGF-2 composite has the potential for hard tissue defect repair.

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Introduction

Mineral trioxide aggregate (MTA) is a biocompatible material and developed by Torabinejad's team in 1993, which has several clinical applications in endodontic treatment, such as root-end fillings, pulp capping, pulpotomy, perforation repairs, and apexification treatment.[1–3] It is a complex mixture of 20% bismuth oxide for radiopacity and similar to Portland cement, which consists of tricalcium silicate (Ca_3SiO_5), dicalcium silicate (Ca_2SiO_4), tricalcium aluminate ($Ca_3Al_2O_6$), tetracalcium aluminoferrite, and 5% gypsum.[4] In previous studies, we found that the newly developed calcium silicate cement (CS) showed an advantageously shortened setting time,[5] excellent bioactivity, and good biocompatibility.[6] Moreover, we recently showed that CS not only stimulates the proliferation and differentiation of human dental pulp cells (hDPCs) *in vitro*,[7–9] but also reduced inflammation *in vivo*.[10]

Several growth factors have been verified as potential therapeutic agents for hard-tissue formation.[11,12] Fibroblast growth factors (FGFs) play an important role in the control of cell adhesion, proliferation, and differentiation in several tissues including bone.[13,14] Notably, FGF-2 has been found to enhance cell proliferation and osteogenic differentiation in bone marrow mesenchymal cells.[15,16] FGF-2 signaling played an important role of in the control of osteoprogenitor cells, and knockout of FGF-2 gene in mice results in decreased bone marrow stromal cell osteogenic differentiation and changed bone formation.[17] FGF binding to FGF receptor (FGFR) leads to receptor phosphorylation of intrinsic tyrosine residues, including mitogen-activated protein kinase and phosphatidylinositol 3-kinase.[18,19] In hard tissue, phosphorylation of extracellular-related kinase, mitogen-activated protein kinases, and protein kinase C have been proved to enhance osteogenic gene expression.[20,21]

In a clinical case, tricalcium phosphate ceramic combined with growth factor and placed in a bone defect after periapical surgery was shown to enhance bone regeneration.[22] It can help regeneration of vital tissue with necrotic pulp and periapical lesions.[22] Growth factors/cytokines are key factors in tissue wound healing and this combination reduced immune function and promoted proliferation and differentiation of cells participating in wound healing.[23] FGF-2 is expressed during tooth development, associated with tooth morphogenesis, and promotes the differentiation of dental pulp cells into odontoblasts in culture.[24] A search of the literature reveals an absence of any publication discussing the cellular effects of the growth factor combined with CS. The aim of the present study was to evaluate CS/FGF-2 composite properties and pulp cell biological effects. It is hypothesized that CS combined with FGF-2 can promote the osteogenesis protein markers expression and help in mineralization.

Materials and methods

Preparation of CS specimens

The CS cement was made according to our previously reported laboratory procedures.[5,25] Appropriate amounts of 65% CaO (Showa, Tokyo, Japan), 25% SiO_2 (High Pure Chemicals, Saitama, Japan), and 5% Al_2O_3 (Sigma-Aldrich, St Louis, MO, USA) powders were mixed by a conditioning mixer (ARE-250; Thinky, Tokyo, Japan). After sintering at 1400°C, the granules were ball milled in EtOH using a centrifugal ball mill (Retsch S 100; Retsch, Hann, Germany) and then dried in an oven. CS cement was mixed according to the liquid/powder ratio of 0.33 mL/g. FGF-2 (ProSpec, Rehovot, Israel) was dissolved in double distilled (dd)H_2O, and the concentration was 3 μg/mL. After mixing, the cement fully covered of the 24-well plate (GeneDireX, Las Vegas, NV, USA) to a thickness of 2 mm The samples were stored in an incubator at 100% relative humidity and 37 °C for 1 day. Prior to the cell experiments, all specimens were sterilized by immersion in 75% ethanol followed by exposure to UV light for 1 hour.

Setting time and strength

After mixing, the cement was placed into a Teflon cylindrical mold (diameter = 6 mm) under a pressure of 0.7 MPa for 1 minute and tested using a 400 g Gillmore needle with a 1 mm diameter, according to International organization for standardization 9917-1 (ISO 9917-1). Six specimens were tested for each measurement.

As for mechanical performance testing, the cement was molded into a cylindrical mold to a diameter of 6 mm and thickness of 2 mm and the specimens were stored in an incubator at 100% relative humidity and 37°C for 24 hours to set. The testing was conducted on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 0.5 mm/minute to obtain diametral tensile strength (DTS).

In vitro release of FGF-2

As for FGF-2 release testing, the cement (0.1 g) was molded into a cylindrical mold to a diameter of 6 mm and thickness of 3 mm and the specimens were stored in an incubator at 100% relative humidity and 37°C for 24 hours to set. The release of FGF-2 was measured by soaking the specimens in 1 mL of Dulbecco's modified Eagle medium (DMEM; Caisson Laboratories, North Logan, UT, USA) at 37°C for several time points. The amount of FGF-2 in DMEM was measured using the FGF-2 enzyme-linked immunosorbent assay kit (ELISA; Invitrogen, Grand Island, NY, USA), and the FGF-2 content following the manufacturer's instructions. All experiments were carried out in triplicate.

Human dental pulp cell isolation and culture

Human dental pulp cells (hDPCs) were derived freshly from a caries free, intact premolar that was extracted for the purpose of orthodontic treatment. The patient provided informed consent, and approval for the study was obtained from the committee of the Chung Shan Medicine University Hospital (CSMUH No. CS11187). The tooth was split sagittally with a chisel and pulp tissue was immersed in phosphate buffered saline (PBS) contained type I collagenase. The tissue was cut into fragments and placed in plates containing DMEM, supplemented with 10% fetal bovine serum (Caisson Laboratories) for 3 days. Cell culture media were supplemented with 100 units/mL penicillin-G, 100 μg/