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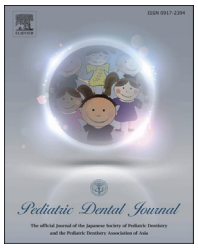

Original Article

Effects of pre-reacted glass-ionomer cement on the viability and odontogenic differentiation of human dental pulp cells derived from deciduous teeth

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ABSTRACT

Surface reaction-type pre-reacted glass-ionomer (PRG) fillers have the ability to slowly release various ions. This study investigated the effects of cement-containing surface reaction-type PRG filler (PRG cement) on the viability and differentiation of human dental pulp cells derived from deciduous teeth (hDPC-Ds). Other materials generally used for pulp capping, Dycal and Fuji glass-ionomer cement Type II, were also tested by culturing hDPC-Ds with the extract of each material. The viability of hDPC-Ds was measured by methyl-thiazol-tetrazolium [3-(4, 5-di-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay. Odontogenic differentiation of hDPC-Ds was evaluated by alkaline phosphatase (ALP) activity and immunocytochemistry. The concentration of each ion in the extract was assessed by ion pair chromatography, inductively coupled plasma, and fluoride ion meter. Based on the results of ion analysis, a medium containing fluoride (F) and aluminum (Al) ions was reconstituted. Human DPC-Ds were cultured in this medium, followed by 3-(4, 5-di-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assays and measurement of ALP activity. PRG cement extracts were found to significantly enhance the viability, ALP activity, ALP staining in the extracellular matrix of hDPC-Ds, and release of F and Al ions. These ions, in turn, enhanced the viability and differentiation of hDPC-Ds. Taken together, these results indicate that the proliferation and differentiation of hDPC-Ds was enhanced by PRG cement and that F and Al, which elute from the PRG filler, were most likely involved in the proliferation and differentiation of hDPC-Ds. These findings suggest that PRG cement is potentially useful as a pulp capping agent.

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1. Introduction

Dental caries of primary teeth are exacerbated more rapidly than caries of permanent teeth. Pulp capping, using calcium hydroxide or glass-ionomer cement, is a standard treatment for dental caries, even those close to dental pulp. Calcium hydroxide has been reported to decrease bacteria and form reparative dentin [1,2]. However, this reparative dentin may be porous and its strong alkalinity [3] has been found to induce inflammation in dental pulp [4]. By contrast, glass-ionomer cement is an anticaries material with a high capacity for slowly releasing fluoride [5,6], but its mechanical strength [7] is insufficient.

Therefore, pre-reacted glass-ionomer (PRG) technology was introduced using an acidic reaction between a surface-modified multifunctional glass (fluoroboroaluminosilicate glass) filler and an aqueous solution of polyacrylic acid, resulting in a stable glass-ionomer phase within the glass filler. This technology led to the development of a surface reaction-type pre-reacted glass-ionomer (S-PRG) filler as a novel bioactive material [8]. S-PRG fillers have been shown to release six types of ion species [9–14], which contribute to various bioactive effects, such as strengthening of tooth structure, antiplaque activity [15–17], remineralization of dentin [18,19], acid-buffering capacity [20,21], and inhibition of enzymatic activity of bacteria [22,23]. S-PRG filler has also been shown to promote HeLa cell proliferation [24]. By releasing various ion species, S-PRG filler can promote the preservation of dental pulp and formation of new dentin, suggesting that this new material may be useful in pulp capping.

Low concentrations of fluoride at levels of 5 ppm [25] and 0.4–1 ppm [26] are useful in the treatment of dental pulp [25,26], gingival fibroblasts [27], and epithelial cells [28], as well as for odontoblasts [29,30], osteoblasts [31–33], and Vero cells [34]. While aluminum ion alone is also involved in osteoblast proliferation [35–37], aluminum and fluoride form a complex, fluoroaluminate, which is a potent inducer of osteoblast proliferation and bone formation [38,39].

Although studies have shown that PRG cement, which contains S-PRG fillers, is a useful material for teeth, its effect on dental pulp remains undetermined. We hypothesized that PRG cement would be extremely useful for pulp capping. Therefore, this study compared the effects of PRG cement with those of traditional pulp capping agents on the proliferation, differentiation, and extracellular matrix mineralization of human dental pulp cells derived from deciduous teeth (hDPC-Ds).

2. Materials and methods

2.1. Culture of hDPC-Ds

Human DPC-Ds were obtained from deciduous teeth that had been extracted during the period of normal exfoliation from patients aged 7–11 years at the Department of Pediatric Dentistry of Kanagawa Dental University Hospital, Kanagawa, Japan. This study was approved by the Ethics Committee of Kanagawa Dental University (No. 229), and all patients and their parents provided written informed consent. After extraction, each deciduous tooth was immediately placed in culture medium, consisting of Dulbecco modified Eagle's medium (DMEM; Gibco, NY, USA), penicillin streptomycin (Gibco), 0.25 mM L-ascorbic acid phosphate magnesium salt n-hydrate (Wako, Japan), and 10% fetal bovine serum (Thermo Scientific, MA, USA). Dental pulp was carefully removed from the pulp chamber of each tooth with a cleanser on a clean bench. Isolated pulp tissue was minced with a surgical knife in a 10 mm culture dish, covered with culture medium, and incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The cells were subsequently collected by trypsinization (Gibco) and subcultured in a fresh medium, which was replaced at intervals of 2–3 days. Human DPC-D cells from the third to seventh passages were used in this study.

2.2. Preparation of extracts

Three filler materials were assessed: PRG cement (Shofu, Japan), Dycal (DY; Dentsply Sankin, Japan), and Fuji glass-ionomer cement Type II (GI; GC, Japan). Powder of each material was mixed with liquids according to the manufacturer's instructions. We performed the experiment as reported by Peng et al. [40]. Extracts of each filler material were prepared by incubating the material in culture medium at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 24 h without agitation, with the extracts subsequently sterilized using a 0.22 μm filter (Millipore, MA, USA). The concentration of each extract was adjusted to 0.5 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL, 25 mg/mL, and 50 mg/mL by adding culture medium, and the extracts were stored at 4 °C until use.

2.3. Cell viability

Cell numbers were determined using 3-(4, 5-di-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Cayman Chemical, MI, USA) assays, according to the manufacturer's instructions. Briefly, hDPC-Ds were seeded at a density of 5.8×10^3 cells per well in 96-well plates and incubated for 24 h. The medium was replaced with a medium containing an extract; the medium without any extract was used as a vehicle control. MTT assays were performed on Days 4, 6, 8, 10, and 12. Alternatively, hDPC-Ds were seeded and incubated for 7 days, and the medium was replaced with a medium containing an extract. MTT assays were performed on Days 9 and 11.

At each time point, the wells were rinsed twice with Dulbecco's phosphate buffered saline (−) (Nissui, Japan), and 10 μL MTT reagent was added to each well. After incubation for 4 h, the solution was removed and a crystal-dissolving solution (100 μL) was added to each well. Absorption of the solutions in the wells at 570 nm was assessed with a microplate reader (iMark Microplate Reader; Bio-Rad, CA, USA).

Human DPC-Ds were seeded at a density of 6.5×10^4 cells per well in 12-well plates in culture medium. At designated time points, hDPC-Ds were collected by trypsinization and their numbers were counted using a hemocytometer to draw a growth curve.