Phytic Acid: An Alternative Root Canal Chelating Agent

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Abstract

Introduction: The objectives of this study were to investigate the effect of phytic acid, inositol hexakisphosphate (IP6), as a final rinse on the surface of instrumented root canals and smear-layered flat dentin surfaces treated with sodium hypochlorite (NaOCl) and to evaluate its effect on the viability and alkaline phosphatase activity of osteoblast-like cells (MC3T3-E1). **Methods:** The universally accepted chelating agent EDTA was used as the control in all conducted experiments. Root canals of human canines were instrumented with rotary files and irrigated with 5% NaOCl, followed by a final rinse of 17% EDTA (1 minute), 1% IP6 (1 minute or 30 seconds), or distilled water. NaOCl-treated flat coronal dentin surfaces were also treated with 17% EDTA (1 minute), 1% IP6 (1 minute or 30 seconds), or distilled water. The presence or absence of smear layer was evaluated with scanning electron microscopy. Cell viability and alkaline phosphatase assays were performed to evaluate the effect of IP6 and EDTA on cultured MC3T3-E1 cells. **Results:** The results demonstrated the ability of IP6 to remove the smear layer from instrumented root canals and flat coronal dentin surfaces. When compared with EDTA, IP6 was less cytotoxic and did not affect the differentiation of MC3T3-E1 cells. **Conclusions:** IP6 shows the potential to be an effective and biocompatible chelating agent. *(J Endod 2015;41:242–247)*

Key Words

Chelating agent, EDTA, phytic acid, root canal, smear layer

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In endodontics there is always a need for chemomechanical debridement (1). Mechanical debridement results in the formation of smear layer on root canal surfaces. According to the literature, removal of the smear layer before obturation is recommended (2). Sodium hypochlorite (NaOCl) is the main irrigant used during root canal treatment (3); however, NaOCl alone cannot effectively remove the smear layer (4). EDTA has been the most commonly used irrigant for this purpose since 1957 (5) in a concentration of 17% and an application time of 1–5 minutes (6). It is most commonly synthesized on an industrial scale from ethylenediamine, formaldehyde, and sodium cyanide. This method results in the formation of impurities that are detrimental to most applications of this chelating agent (7). This synthetic persistent material is being overused and is considered one of the major organic pollutants discharged in water (8). It is noteworthy that EDTA is used in cosmetic formulations in a concentration less than 2% (9). Because EDTA is not readily biodegradable, there have been some concerns about the leakage of this irrigant into the periapical tissue. Because of these concerns, the extrusion of EDTA beyond the root canal should be avoided (10, 11). Considering these facts, an alternative agent for smear layer removal is warranted, and the search for more biocompatible material to replace EDTA is still going on.

Phytic acid (IP6, inositol hexakisphosphate) is the major storage form of phosphorus in plant seeds and bran that contributes in a variety of cellular functions (12). It is also omnipresent in mammalian cells, with a concentration ranging from 10 to 100 μmol/L (13, 14). IP6 can be extracted with low cost from rice bran (15). This agent has multiple negative charges, making it an effective chelator of multivalent cations such as calcium (Ca^{+2}), magnesium, and iron (16, 17). On the basis of these properties, we speculate IP6 to have the potential to replace EDTA as a root canal chelating agent. Thus, the aims of this study were (1) to determine the efficacy of IP6 in removing the smear layer on NaOCl-treated flat coronal dentin surfaces and instrumented root canals dentin and (2) to assess IP6 effect on the viability and alkaline phosphatase (ALP) activity of osteoblast-like cells (MC3T3-E1).

Materials and Methods

Smear Layer Removal Effect

Flat Coronal Dentin Surface. Human non-carious third molars were used in this part of the study. Flat coronal dentin surfaces of 1-mm thickness were created perpendicular to the tooth's longitudinal axis by using a slow-speed diamond saw (Isomet Low Speed Saw; Buehler, Lake Bluff, IL) under water lubrication. A smear layer was created on each surface by using 600-grit silicon-carbide paper under water irrigation. The control specimens received only a rinse with distilled water, whereas the other specimens were treated with 5% NaOCl (pH 12) (Wako Pure Chemical Industries, Osaka, Japan) for 5 minutes, followed by 17% EDTA (pH 7.5) (Wako Pure Chemical Industries) for 1 minute, 1% IP6 (pH 1.3) (Wako Pure Chemical Industries) for 1 minute or 30 seconds, or distilled water. All solutions were applied with agitation by using a microbrush. After rinsing with distilled water for 10 seconds, specimens were dehydrated with ascending concentrations of ethanol (25%, 50%, and 75% for 20 minutes, 95% for 30 minutes, and 100% for 60 minutes), followed by immersion in hexamethyldisilazane (Wako Pure Chemical Industries). Specimens were dried overnight inside a covered glass vial and then sputter-coated with gold/palladium and observed under a scanning electron microscope (SEM) (JSM-5310LV scanning microscope; JEOL, Tokyo, Japan) operating at 5 kV.

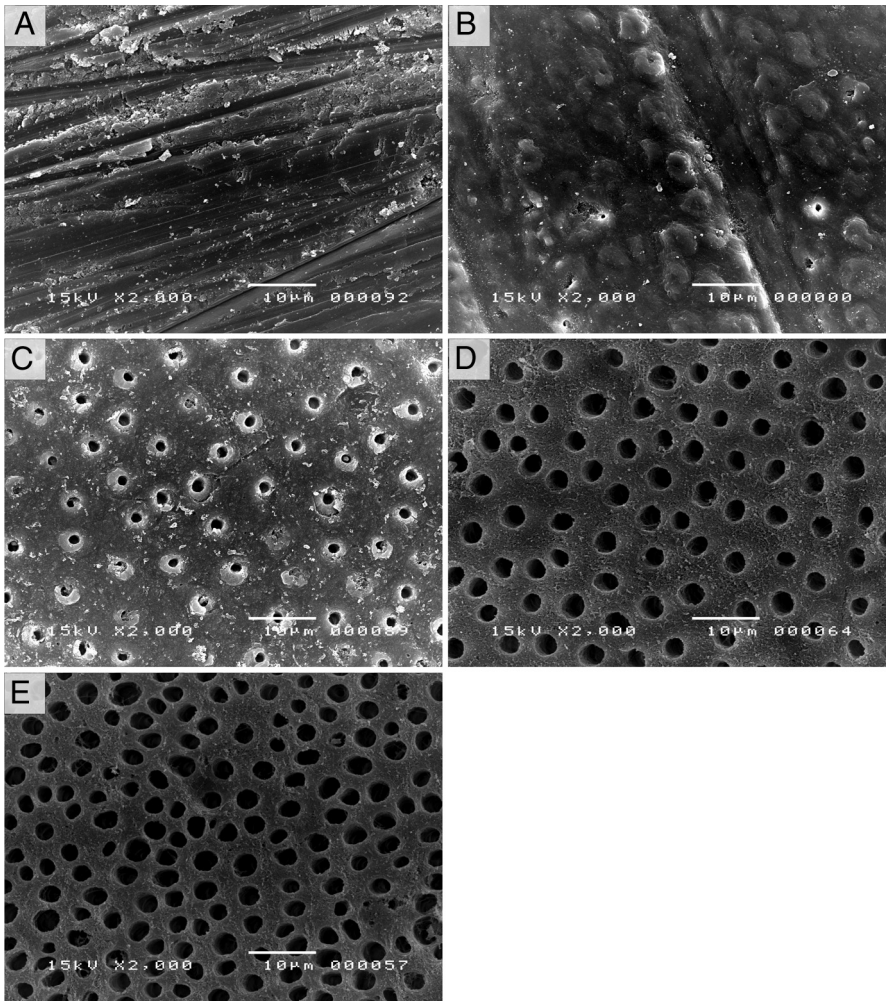

Figure 1. Representative SEM images of effect of different treatments in removing the smear layer from flat coronal dentin surfaces. (*A*) Smear layer produced by 600-grit silicon-carbide paper. (*B*) 5% NaOCl applied for 5 minutes. NaOCl was ineffective to completely remove the smear layer. (*C*) 17% EDTA applied for 1 minute on NaOCl-treated flat coronal dentin surface. EDTA resulted in clean dentinal surface with open dentinal tubules. (*D* and *E*) 1% IP6 applied for 1 minute or 30 seconds, respectively, on NaOCl-treated flat coronal dentin surface. IP6 resulted in clean and debris-free surface with widely open dentinal tubules.

Root Canal Surface. Single-rooted maxillary human canines with straight roots and mature apices were used in this part of the study. The experimental setup was followed according to the closed system proposed by Tay et al (18). ProTaper nickel-titanium rotary instruments (Dentsply Maillefer; Ballaigues, Switzerland) were used to prepare and shape the canals according to the manufacturer's instructions, ending with F3 file. Between each file, the canals were irrigated with 1 mL 5% NaOCl. The canals were rinsed with distilled water before the application of the chelating agents. A final rinse of 1 mL 17% EDTA for 1 minute or 1 mL 1% IP6 for 1 minute or 30 seconds was performed with agitation by using a hand #15 K-file. The canals were then irrigated again with distilled water and dried with absorbent paper points. The control group received no treatment after instrumentation except for a final rinse with distilled water. To facilitate the separation of the root into halves, deep longitudinal grooves were prepared on the external root surface, followed by splitting the root by using a hammer and chisel. The dehydration process for SEM observation was conducted in the same previously mentioned manner. Representative images of the middle and apical thirds at $\times 1000$ magnification were taken for each group.

Effect on Cell Viability and ALP Activity

Cell Viability Assay. The clonal cell line (MC3T3-E1), osteoblast-like cells, established from mouse calvaria, was used in the present study. To each well of 24-well culture plates, MC3T3-E1 cells (5×10^4 cells/well) were placed and incubated for 24 hours in 5% CO_2 incubator at 37°C. Six wells were allocated for each test solution. The test solutions included various dilutions (500–10,000 μg/mL culture medium) of either 17% EDTA or 1% IP6. An aliquot of 300 μL of each experimental solution was added to each well and incubated in 5% CO_2 incubator at 37°C for 24 hours. Cell culture in fresh medium without experimental solution served as the control. After the incubation time, culture medium was discarded, and cells were washed with 200 μL phosphate buffer solution to prevent any interaction between the test solutions and the colorimetric assay. One hundred microliters of new culture medium