Evaluation of the Effects of Endodontic Materials on Fibroblast Viability and Cytokine Production

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Introduction: Recently, a new sealer composed of Portland cement named Endo-CPM-Sealer was developed. The aim of this study was to investigate the effects of Endo-CPM-Sealer (EGEO SRL, Buenos Aires, Argentina), Sealapex (Sybron Endo, Glendora, CA), and Angelus MTA (Angelus, Londrina, Brazil) on cell viability and cytokine (interleukin [IL]-1β and IL-6) production by mouse fibroblasts. Methods: Millipore culture plate inserts with polyethylene tubes filled with materials were placed into 24-well cell culture plates with mouse fibroblasts. Cells cultured with only empty polyethylene tubes were used as the control. After 24 hours, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay was used to evaluate the cell viability. For cytokine assay, mouse fibroblasts were incubated in 24-well flat-bottom plates with set material disks at the bottom. Cells cultured without the material disks served as the negative control. After 24 hours of incubation, culture media were collected for cytokine evaluation by using an enzyme-linked immunosorbent assay. The data were statistically analyzed by analysis of variance and Bonferroni correction. Results: Endo-CPM-Sealer, Sealapex, and Angelus MTA did not inhibit the cell viability. All materials induced IL-6 releasing, but the amount was not statistically significant compared with the control group. Angelus MTA induced IL-1β releasing significantly more than the control. Conclusions: All materials were not considered cytotoxic in fibroblast culture. (J Endod 2009;35:1577–1579)

Key Words
Cell culture, cytokine, cytotoxicity, endodontic materials

The ideal endodontic material should be biocompatible, dimensionally stable, capable of inducing osteogenesis and cementogenesis, easy to prepare and to use, sterile, radiopaque, and inexpensive (1, 2).

Mineral trioxide aggregate (MTA) was introduced to be used in pathologic or iatrogenic root perforations and in root-end cavities (2, 3). Studies have shown that MTA promotes favorable tissue reactions that are characterized by the absence of severe inflammatory response, the presence of a fibrous capsule, and the induction of formation of mineralized repair tissue (4–6). However, MTA has working properties that are less than ideal. The resulting cement from the mixing of powder with water is difficult to manipulate (7), and its setting time has been reported to be almost 3 hours, whereas the working time is less than 4 minutes (8).

Sealapex (Sybron Endo, Glendora, CA) is a calcium hydroxide–containing sealer clinically accepted mainly because of the healing process obtained from its use (9, 10). The diffusion of calcium and hydroxyl ions from the sealer raises the pH at the surface of the root adjacent to periodontal tissues. It also favors the repair (11), the antimicrobial action (12), the degradation of bacterial lipopolysaccharides (13), the induction of hard tissue formation (14), and also the control of inflammatory root resorption (15, 16).

A new formulation of Portland cement labeled Endo-CPM-Sealer (CPM Sealer; EGEO SRL, Buenos Aires, Argentina) was created to be used as root canal sealer. The composition of CPM Sealer after mixing is mineral trioxide aggregate (SiO2, K2O, Al2O3, SO3, CaO, and Bi2O3: 50%; SiO2: 7%; CaCO3: 10%; Bi2O3: 10%; BaSO4: 10%; propylene glycol alginate: 1%; propylene glycol: 1%; sodium citrate: 1%; and calcium chloride: 10% according to the manufacturer). The chemical composition is similar to the MTA, but it has the addition of calcium carbonate to reduce the pH from 12.5 to 10.0 after set.

There are some experimental models used to evaluate the biocompatibility of endodontic materials such as cell culture (17), which has the advantage of being relatively inexpensive and rapid (18) and can be determined with reality and reproducibility (19). However, there has been no study in the literature evaluating cell viability and cytokine production induced by Endo CPM Sealer. Thus, the aim of this study was to determine the effects of the Endo-CPM-Sealer, Sealapex, and Angelus MTA on cell viability in fibroblasts and to assess the effects of these materials on the releasing of IL-6 and IL-1β.

Material and Methods

Cell Culture

L929 mouse fibroblasts were grown in Dulbecco Modified Eagle’s Medium supplemented with 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD) streptomycin (50 g/mL), and 1% antibiotic/antimycotic cocktail (300 U/mL, 300 µg/mL streptomycin, 5 µg/mL amphotericin 100 g/mL) (GIBCO BRL, Gaithersburg, MD) under standard cell culture conditions (37°C, 100% humidity, 95% air, and 5% CO2).

Test Material

The materials used in this study were Endo-CPM-Sealer (Egeo SRL, Buenos Aires, Argentina), Sealapex, and Angelus MTA (Angelus, Londrina, Brazil). The materials tested were prepared according to the manufacturers’ recommendations.
Cytotoxicity Testing

L929 fibroblasts were seeded into the 24-well plates (3 x 10^4 cells/1 mL medium per well). The cells were incubated for 24 hours in a humified air atmosphere of 5% CO_2 at 37°C. The test materials were placed in polyethylene tubes (BARD, C.R.; Bard Ireland LTDA, Galway, Ireland) with a 1.1-mm inner diameter and 10-mm length and inserted into the fibroblast culture. Six wells were used for each material, and an empty tube was used as the control. The exposures of cell cultures were stopped by the discarding of the exposure mediums after 24 hours. Viable cells were stained with formazan dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) (Sigma Chemical Co, St Louis, MO). MTT was dissolved in phosphate-buffered saline at 5 mg/mL and filtered in order to sterilize and remove a small amount of insoluble residue. At the times indicated later, stock MTT solution (20 μL per 180 μL medium) was added to all wells of an assay, and plates were incubated at 37°C for 4 hours. The medium was then removed by the inversion of the plate and the dumping of 200 μL of isopropyl alcohol, which was added to the wells and mixed during 30 minutes in order to dissolve the dark blue crystals. The blue solution was transferred to a 96-well plate, and the absorbance was read in the microplate reader by use of a test wavelength of 570 nm (20).

Cytokine Assay

For cytokine assay, the tested materials were inserted into the wells of 24-well flat bottom plates (corning) and condensed to disks that were approximately 1-mm thick and with the same diameter of the wells. The material was allowed to set for 2 weeks in cell culture medium at 37°C. The medium was changed every day during this time. L929 fibroblasts were seeded into the wells (10^6 cells/1 mL medium per well) with the material disks in the bottom. The plates were incubated for 24 hours. After incubation, culture media were collected and analyzed for IL-1β and IL-6 content by ELISA (R&D Systems, Inc, Minneapolis, MN). Cells cultured without tested material served as negative controls.

Statistical Analysis

The results were statistically analyzed by analysis of variance with Bonferroni correction (P < 0.05).

Results

ELISA assay revealed that the average of IL-6 (pg/mL) releasing was higher when cells were cultured in the presence of Endo-CPM-Sealer, Sealapex, and Angelus MTA than in the control for 24 hours but with no statistically significant difference (Fig. 1A). The release of IL-1β was statistically and significantly higher than the control only for the Angelus MTA (Fig. 1B). After 24 hours, Endo-CPM-Sealer, Sealapex, and Angelus MTA produced a mild cytotoxic effect, but they did not inhibit the cell viability (Fig. 1C).

Discussion

Endodontic materials should be biocompatible and have satisfactory physicochemical properties. The toxic effect of materials used for endodontic therapy are of particular concern once they can cause degeneration of the periapical tissue and delay wound healing (21). In this study, the cell viability was determined by MTT assay based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium salt MTT into dark blue formazan crystals. The advantages of this method are the simplicity, rapidity, and precision. In addition, it does not require radioisotopes

Figure 1. (A) Mean levels of IL-6 were raised when the cells were grown in the presence of the materials. There was no statistically significant difference (P > 0.05) between the experimental materials and the control group. (B) There was a statistically significant difference (P < 0.05) only between MTA and the control group. (C) Viability of fibroblasts was not statistically different (P > 0.05) between the experimental materials and the control group. These results were expressed as means of the absorbance (A570nm) ± standard deviation of each material and the control group.

(20, 22). Statistical analyses of the data of the MTT assay showed no significant difference among the three cements in 24 hours. MTA has been recommended to seal all pathways of communication between the root canal system and the external surface of the tooth. The principal components of MTA include tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide (2). The results in the present study agree with previous ones that showed that MTA was not cytotoxic (23–26).

Sealapex was used in this study as a reference of calcium hydroxide–containing root canal sealer because of the presence of numerous studies in the literature (11–16) and the clinical acceptance based on the favorable healing process observed with its use (9, 10). This material was not cytotoxic compared with the control group. The results of this study disagree with other previous studies that proved Sealapex to be cytotoxic (27–29), perhaps because of the high pH after
The synthesis of cytokines is complex, and their expression and effects are governed by many factors including other cells and chemical mediators (30). Previous studies showed that MTA stimulated the production of IL-1β by osteoblasts (31–33). IL-1β is a cytokine that mediates the bone resorption. It is synthesized by various cells and macrophages close to the bone resorption and the osteoclasts (34). In this study, all materials induced IL-1β releasing, but only the Angelus MTA was statistically significant. IL-6, on the other hand, is a cytokine that mediates the host response to injury and infection, and it is secreted during the inflammatory process in order to regulate various aspects of the immune response, the acute phase of the reaction, and the control of hematopoiesis infection (35). Animals depleted of IL-6 showed larger the immune response, the acute phase of the reaction, and the control of during the inflammatory process in order to regulate various aspects of that mediates the host response to injury and infection, and it is secreted.

It was possible to conclude that Endo-CPM-Sealer, Sealapex, and Angelus MTA did not inhibit 1929 fibroblasts viability. Moreover, Angelus MTA significantly induced IL-1β releasing, but no material significantly induced IL-6 releasing.

References