Cytotoxic Effects of Primary Tooth Root Canal Filling Materials on L929 Cell Line

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ABSTRACT

Purpose: The purpose of this study was to determine the cytotoxic effects of five different primary tooth root canal filling materials on L929 permanent cell line with MTT assay.

Methods: Kri 1 paste (iodoform), Diapex (iodoform+$Ca(OH)_2$), Metapaste ($Ca(OH)_2$ with distilled water), Dentalis (iodoform+ZOE+$Ca(OH)_2$) and Kalsin ($Ca(OH)_2$ with glycerin) were used in this study. Tested materials were in contact for 24, 48 and 72 hours with L929 cells. At the end of the test periods, MTT test solutions were added to the plates and incubated for 3 hours at 37°C. Then optic densities were read using UV visible spectrophotometer. All assays were repeated three times to ensure reproducibility. The obtained data were analyzed statistically by one-way analysis of variance and Dunnett T3 post hoc test ($P<0.05$).

Results: All tested materials were found cytotoxic on L929 cell line. It was found that Kri 1 paste group showed the highest survival rates.

Conclusions: We concluded that the use of Kri 1 paste as a root canal filling material is a better option than other medications in primary teeth. Further research is necessary to determine the effect of root canal filling materials on vital tissues.

(J Dent Child 2010;77:72-76)

Received February 21, 2009; Last Revision March 29, 2009; Revision Accepted March 30, 2009.

KEYWORDS: CALCIUM HYDROXIDE, IODOFORM, ZINC OXIDE EUGENOL, L929, CYTOTOXICITY

Pulpectomy is a root canal procedure for pulp tissue that is irreversibly infected or necrotic due to caries or trauma. The main objective of pulp treatment in primary teeth is to maintain the integrity and health of the oral tissues and permanent teeth by filling the root canals with resorbable materials after the removal of necrotic pulp tissue.[9]

A number of different materials have been reported to be suitable for use as filling materials in root canal treatment in primary teeth. The traditional root canal filling materials for primary teeth are calcium hydroxide (**$Ca(OH)_2$**), zinc oxide (**ZnO**), zinc oxide eugenol (**ZOE**) with or without mixed with formocresol; iodoform paste, such as Kri 1 paste (a mixture of iodoform and parachlorophenol, camphor, and menthol); or Diapex (a mixture of iodoform, $Ca(OH)_2$, and silicone).[7,23]

Animal studies using ZOE as a root canal filling material have reported chronic inflamatory reactions and slow resorption of the material.[11] It also has been noted that ZOE irritated the periapical tissues and caused necrosis of the bone and cementum.[5,8] Unfavorable responses of periapical tissue to iodoform paste and increased cytotoxicity have been reported.[25] $Ca(OH)_2$-based filling materials showed mild to moderate tissue-irritating activities.[18]

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An ideal root canal filling material for primary teeth must have several properties, such as resorbing at a rate similar to that of the primary root, being harmless to the periapical tissues and permanent tooth germ, resorbing readily if pressed beyond the apex, and being strongly antiseptic.[18] Indeed, since the material will be in direct contact with periapical tissues for prolonged periods of time, their biocompatibility is of primary importance.[11]

Biocompatibility is defined as the ability of a material to function in a specific application in the presence of an appropriate host response.[10] Biocompatibilty of these endodontic materials is characterized by many parameters such as genotoxicity, mutagenicity, carcinogenicity, cytotoxicity, histocompatibility, or microbial effects.[14] Cell culture studies have been performed for more than 30 years to investigate the cytotoxic reactions. Permanent cell lines (eg, HeLa, 3T3 or L929 cells, and primary cells, mainly oral fibroblasts) are used for these experiments.[2,3]

The aim of this study was to compare the cytotoxic effects of five different primary tooth root canal filling materials on a L929 permanent cell line.

METHODS

CELL CULTURE

The L929 mouse fibroblast cells used for these experiments were obtained from the Institute of Foot and Mouth Disease, Animal Cell Culture Collection, Ankara, Turkey. The cells were grown as a monolayer culture in Dulbecco's Modified Eagle's medium/Ham's Nutrient Mixture F-12 (DMEM, Biochrom, Berlin, Germany). They were supplemented with 10% fetal bovine serum (FBS; Biochrom, Berlin, Germany), 1% L-glutamine (Biochrom), 100 IU/mL penicillin (Biochrom), and 50 µg/mL strepto-mycin (Biochrom) at 37°C in air containing 5% carbon dioxide and 95% relative humidity. Cells from the fourth collection were plated in a 96 well plate at a density of 5×10^3 cells per well and allowed to attach for 24 hours to the DMEM plus supplements.

To obtain sufficient amount of cells for the experiments, cells were passaged by treatment with trypsin (0.25%) and ethylene diamine tetra-acetic acid (2%; Biochrom) and incubated at 37°C. Cultures were examined after 24, 48, and 72 hours had been seeded with 10^5, $5x10^4$, and 10^4 cells/ml, respectively, and incubated overnight at 37°C in air containing 5% carbon dioxide and 95% relative humidity.

PREPARING THE ROOT CANAL FILLING MATERIALS

The tested materials, product names, manufacturers, and ingredients are listed in Table-1. The root canal filling materials were mixed according to the manufacturer's in-structions under aseptic conditions in vertical laminar flow cabin (Heraeus, Berlin, Germany) and placed into polyte-traphloroethylene polymer rings (4-mm diameter, 4-mm height) for the standardization of the amount of root filling materials. Polytetraphloroethylene polymer rings without adding any material were used as a control. The samples were placed for 2 hours under ultraviolet light in vertical laminar flow cabin to sterilized experimental materials.

EXPOSURE OF L929 CELLS TO MATERIALS

After overnight attachment of the L929 cells, cell culture inserts placed over wells and sterilized samples placed over the inserts measuring 10 mm in diameter (Nunc Cell Culture Inserts, Roskilde, Denmark). The insert contained a permeable membrane (0.4 µm-pore size) and was used to prevent direct physical interaction between the cells and the specimens while allowing for soluble compounds from the specimens to reach the cells.

MTT ASSAY

The cytotoxic effect of root canal filling materials were measured by colorimetric assay called MTT assay that was developed by Mosmann.[17] This assay measures the conversion of a yellow water-soluble MTT dye into a purple formazan product by active mitochondria via an electron current. MTT solution was prepared in 5 mg/ml of phosphate-buffered saline just before use and filtered through a 0.22 µm filter.

After 24, 48, and 72 hours, root canal filling materials were removed from the wells and 1 ml MTT test solution, prepared with cell culture medium (proportion=1:9) was added to the wells for 3 hours. On termination of the experiment, the entire medium was discarded by inverting and tapping the plates and 1 ml dimethyl sulfoxide (DMSO; Biochrom) was added to each well. Subsequently, the absorbance at 570 nm was measured using a UV-visible spectrophotometer (Molecular Device Counterpane, Inc. Washington, USA). Five replicates of each concentration were performed in each test. All assays were repeated 3 times to ensure reproducibility.

Table 1. Product Names, Manufacturers, and Ingredients of the Tested Materials

Product name	Manufacturer	Ingredients
Metapaste®	META Biomed Co, Ltd, Korea	$Ca(OH)_2$, barium sulfate, distilled water
Kalsin®	Aktu Tic Ltd, Izmir, Turkey	$Ca(OH)_2$, barium sulfate, glycerin
Kri 1 paste®	Pharma Chemre, Haarlem, The Netherlands	Iodoform (80%), parachlorophenol (2%), camphor (5%), menthol (1%), others
Diapex®	DiaDent Group International Inc, Vancouver, Canada	$Ca(OH)_2$ (30%), iodoform (40%), silicone oil (23%), others (7%)
Dentalis®	Neo Dental International Inc, Tokyo, Japan	ZOE, iodoform, $Ca(OH)_2$

STATISTICAL ANALYSIS

Mean absorbance values obtained from the DMSO-solubilized formazan for each specimen were calculated and expressed as a percentage of the mean control value (set at 100% viability). Differences in mean cell viability values between materials were assessed by one-way analysis of variance and Dunnet T3 posthoc test (P<.05).

RESULTS

Figures 1 and 2 show the cytotoxic effects of the 24-, 48-, and 72-hour elutes on a culture system. It was found that Kri 1 paste was the least toxic root canal filling material. Seventy-two-hour elutes were more toxic that 24- and 48-hour elutes.

DISCUSSION

The properties required of an ideal root canal filling material are well established. No currently available material, however, meets all necessary criteria. The purpose of this study was to evaluate the cytotoxicity of various types of primary tooth root canal filling materials on a permanent cell line. All tested materials were found cytotoxic. Three-day elutes were more toxic than 1-day elute in all tested material groups. This was in accordance with a previous report.[26]

Successful endodontic treatment requires technique that respect apical and periapical tissues and which is completed with filling of the root canal with inert, dimensionally stable, and biocompatible substances.[6] Because these materials are in direct contact with apical and periapical tissues for a prolonged period of time, biocompatibility is of great importance.[3,15]

The employment of in vitro tests offers the possibility of studying the effect of the release of material components on cell systems.[21] Cell culture studies have been used for more than 30 years for investigation of cytotoxic reactions induced by endodontic materials.[24] Continuous cell lines like L929 mouse fibroblasts are being routinely used for the testing of cytotoxic properties of dental materials because of their reproducible growth rated and biological responses.[26]

The MTT assay focuses on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium salt 3,4 (4,5-dimethylthiazol-2-yl) -2,5-diphenylte trazolium bromide (MTT) into dark-blue formazan crystals. The water insoluble product is stored in the cytoplasm of living test cells. The amount of formazan formed is directly proportional to the mitochondrial enzyme activity in a given cell line.[21]

Biological compatibility of materials used in dentistry is of special interest because the toxic ingredients present in these materials could produce irritation or even degeneration of the surrounding tissues. Materials used as root canal filling material must be well tolerated by the surrounding tissues.[27]

Zinc oxide eugenol has been the material of choice for many years. Several investigations regarding the cytotoxicity of root canal sealers showed that zinc oxide-

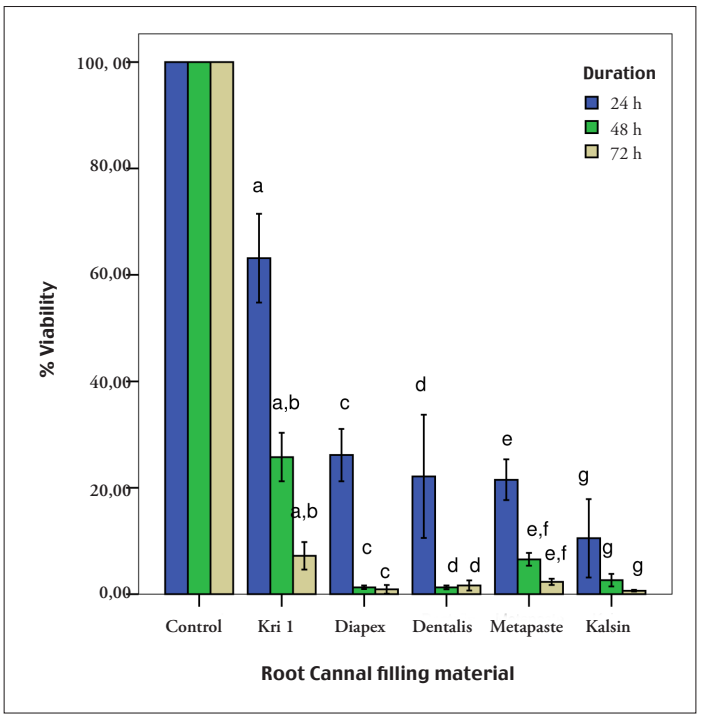

Figure 1. The viability of cells according to the root canal filling materials. a, b, c, d, e, f, g indicate significance between the groups (P<.05).

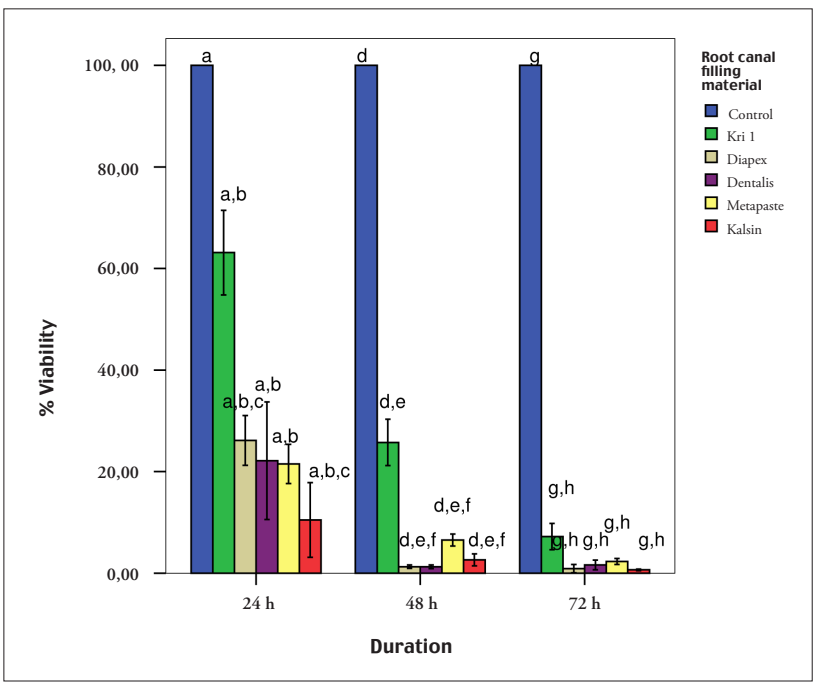

Figure 2. The viability of cells according to duration. a,b,c indicate significance between the groups at 24 hours durations (P<.05), and d,e,f and g,h indicate significance between the groups at 48 hours duration (P<.05).

eugenol sealers have a relative higher cytotoxicity; thus, $Ca(OH)_2$-based sealers have gained popularity due to their biological compatibility.[1,16] Eugenol is a phenol derivative and has been reported as a toxic component in this type of sealer.[17] A case has been reported of arrested tooth formation after zinc oxide-eugenol/formocresol paste was extruded from the apex of a primary tooth pulpectomy.[2]

$Ca(OH)_2$ in an aqueous vehicle has been shown to be the best and most effective intracanal antibacterial agent.[4] $Ca(OH)_2$ paste is a slow-acting antimicrobial agent, and an in vitro study suggests that at least 1 day is required before a full antimicrobial effect is produced.[25]

Contrary to the antibacterial effects, Vitapex and $Ca(OH)_2$+H_2O_2 showed good biocompatibility in a previous investigation.[11] Thus, the addition of strong antibacterial medicine does not meet the basic requirements of the root canal treatment. When primary root filling materials have strong antibacterial properties, the cytotoxicity is strong as well.[13]

It was reported that iodoform-based root canal filling materials caused considerable tissue necrosis and had a higher cytotoxicity than ZOE. Also, it has been reported that the use of iodoform or its combinations caused allergic reactions in some individuals.[28] This is contrary to our present result, which showed the highest survival rate in Kri 1 paste groups. Huang et al' study, however, agreed with our results.[13] It is difficult or even impossible to compare the results from different cell culture experiments because of the many variations in experimental conditions such as the cell type, cell-material contact method, and exposure time.

CONCLUSIONS

Using Kri 1 paste as a root canal filling material in primary tooth root canals may result in a more favorable response. Although in vitro screening tests are very helpful to assay the biological effects of dental materials, they are limited in their ability to simulate the clinical condition. Thus, it is impossible to biologically characterize the materials by a single test method alone, and their properties need to be investigated by a battery of various in vitro and in vivo tests in a structured approach.

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