

In Vitro Cytotoxic Effects of Different Endodontic Pastes Used in Pediatric Dentistry

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• Conflicts of interest: none declared.

ABSTRACT

Objective: The aim of the present study was to evaluate the cytotoxic potential of three different endodontic filling materials on human osteoblast cell cultures. **Material and Methods:** The endodontic pastes Calen PMCC (SSWhite Dental Articles LTDA, Rio de Janeiro, Brazil), Feapex (Formula and Action, São Paulo, Brazil) and CTZ (Lenzafarm, Belo Horizonte, Brazil) were eluted in cell culture medium during 24 hours in an oven at 37°C and 5% CO₂. Four distinct dilutions of these media were performed at the concentrations 1:1, 1:2, 1:4 and 1:8. Cell cultures of Saos-2 Human Osteoblast-like were exposed to these dilutions for 24 hours. The negative control group was performed by exposing the cells to culture medium without contact with any endodontic paste. The cytotoxic potential of these media was evaluated using the MTT assay and the results were transformed into viable cell percentages in relation to the negative control group. Statistical analysis was submitted with a significance level of 5%, using univariate Analysis of Variance (ANOVA) followed by Tukey's test. **Results:** Cell viability was significantly altered according to the material tested ($p < 0.05$) and its concentration ($p < 0.05$). Feapex samples presented higher cell viability than the other materials in all concentrations tested ($p < 0.05$). Although no statistically significant difference was observed between Calen PMCC paste and CTZ paste at concentrations of 1: 1 and 1: 2 ($p > 0.05$), CTZ paste showed a higher cytotoxicity at concentrations of 1: 4 and 1: 8 ($p < 0.05$). In general, cytotoxicity decreases with increasing material dilution. **Conclusion:** According to the results of the present study, Feapex endodontic paste seems to be the better option for use among the analyzed pastes, since it presented lower cytotoxicity than Calen PMCC and CTZ pastes.

Keywords: Root canal filling materials; Endodontics; Deciduous teeth.

Introduction

One of the great clinical challenges in pediatric dental care is the maintenance of the primary teeth under healthy conditions until it reaches their physiological exfoliation.¹ The premature loss of the dental element can result in harmful consequences, such as loss of space for the eruption of the permanent tooth and many problems related to phonetics, masticatory function and aesthetics.^{1,2} When there is a need for intervention in the root canal in order to treat and maintain the deciduous tooth in function, the biological reaction of the pulp and periapical tissues is similar to what occurs on the permanent tooth, except for the physiological process of root resorption.³

One of the main obstacles in pulp therapy of primary teeth is related to the root canal filling, since the physiological root resorption can enable that the filling material causes damage to the periodontium and germs of the permanent tooth.⁴ Therefore, an ideal endodontic filling material for primary teeth must be resorbable continuously with the root resorption and present a low toxicity to the periapical tissues.⁵

Among the filling materials available, the most indicated in pediatric dentistry are the pastes based on zinc oxide and eugenol (ZOE), based on calcium hydroxide and the ones that contain iodoform in their formulation.⁶ The ZOE pastes have restricted antimicrobial activity, a slower rate of resorption than the roots of the primary teeth and are irritating to the periapical tissues, triggering an inflammatory foreign body reaction.^{5,7} As a consequence, its use has been decreased in the last years.⁸

Calcium hydroxide-based pastes are considered biocompatible, act in the formation of mineralized tissue and have an adequate degree of resorption.⁵ They also have good antimicrobial action due to their high pH and the release of hydroxyl and calcium ions.⁹ In turn, the Feapex paste (Formula and Action, São Paulo, Brazil), formed by the addition of iodoform to calcium hydroxide, is more radiopaque and very biocompatible, but presents a higher potential of resorption than the roots of primary teeth.¹⁰

In addition to these pastes, an endodontic paste composed of chloramphenicol, tetracycline and ZOE (CTZ paste) has also been indicated as an alternative to fill the root canals of primary teeth.¹¹ The application of the CTZ paste can be performed without the instrumentation of the root canals, making it easily to use in pediatric patients.^{12,13} There are few studies regarding the biocompatibility of this paste and the results are still conflicting. Studies in animal models showed a severe inflammatory response in the periapical tissues after the use of the CTZ paste.^{3,13} One of them observed that this response remained even after 12 weeks of use³, however, another study showed that the paste can become biocompatible after 63 days of implementation¹⁴. In view of the inconsistency of results, a better elucidation of the biological effects of the CTZ paste becomes necessary. Thus, the objective of the present study was to evaluate the cytotoxicity of the CTZ paste in comparison to the Calen PMCC and Feapex pastes. The null hypothesis is that there is no difference in the cytotoxic effects of the different pastes tested.

Material and Methods

Preparation of the endodontic pastes

The pastes used were: Calen PMCC (SSWhite Dental Articles LTDA, Rio de Janeiro, Brazil), Feapex (Formula and Action, São Paulo, Brazil) and CTZ (Lenzafarm, Belo Horizonte, Brazil), each one handled according to its manufacturer instructions. Disks of each material were prepared under aseptic conditions in a sterile mold (O'ring) according to the ISO 6876:2012, with 5.0 mm of diameter and 2.0 mm thickness.¹⁵ The molds were stored during 24 hours in an oven at 37°C and 5% CO₂. After this period, the O'rings were stored in Dulbecco's Modified Eagle Medium (DMEM) (PAA, Pasching, Germany) containing 10% fetal bovine serum, penicillin and streptomycin in an oven at 37°C and 5% CO₂ for 24 hours. A ratio between the surface of the endodontic paste and the volume of the medium of approximately 150 mm²/ ml was used.

Cell culture

The cells used in this study were the Human Osteoblast-like Saos-2 ("Sarcoma osteogenic") initially grown in DMEM medium with 10% fetal bovine serum, penicillin and streptomycin in oven at 37°C and 5% CO₂. The experiment was carried out in triplicata, using a total of 1x10⁵ cells/well in a 96-wells plate, using a suspension of 200 µl of culture medium, incubated for 24 hours in oven at 37°C and 5% CO₂.

Cell exposure to the extract

After the 24-hour setting time of the endodontic pastes, four serial dilutions were performed with the incorporation of the DMEM culture medium on the extract of the pastes, as follows: 1:1 and 1:2 (50% of the extract), 1:4 (25% of the extract) and 1:8 (12.5% of the extract).

The cells were exposed to the diluted extracts from the endodontic pastes during a period of 24 hours, incubated in oven at 37°C and 5% CO₂. The negative control was performed by exposing the cells only to culture medium, without contact with the endodontic paste.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay - Colorimetric assay

The cytotoxicity test was performed in accordance with the international standard ISO 6876:2012. After the period of contact of the cells with the paste extract, the medium was aspirated, the cells were washed twice with sterile PBS and 100µl of the MTT solution (0.5 mg/ ml) was added to all wells. After incubation for two hours, all MTT solution was removed and 100µl of pure DMSO was added. The plate was agitated for 5 minutes and then remained immobile for 5 minutes for color stabilization. The analysis was performed in a Biotek-Epoch spectrophotometer (Winooski, VT, USA) with absorbance of 540 nM.

Statistical analysis

Analysis of Variance (ANOVA) was applied to examine differences in the percentages of cytotoxicity between groups

using the SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). Tukey's Post Hoc multiple comparison test was used to isolate and compare the results using a significance level of 5%.

Results

The MTT assay showed that the cell viability was significantly altered according to the material tested ($p < 0.05$) and to the concentration of the extract ($p < 0.05$). In all tested concentrations, Feapex showed greater cell viability than the other tested materials ($p < 0.05$). Although no statistically significant difference was observed between the Calen paste and the CTZ paste at concentrations of 1:1 and 1:2 ($p > 0.05$), the CTZ paste showed greater cytotoxicity at concentrations of 1:4 and 1:8 ($p < 0.05$). In general, cytotoxicity decreased with increasing material dilution. The results are presented in Table 1.

Table 1. Cell viability in different concentrations of the tested materials. The results show the percentage average and standard deviation calculated according to the negative control (cells not exposed to the material).

	1:1	1:2	1:4	1:8
Calen	5.2±0.6 ^{Aa}	5.1±0.6 ^{Aa}	37.7±8.1 ^{Bb}	63.0±8.0 ^{Bc}
CTZ	6.5±0.8 ^{Aa}	4.7±0.2 ^{Aa}	4.4±0.3 ^{Aa}	38.4±10.5 ^{Ab}
Feapex	56.1±7.6 ^{Ba}	70.6±10.7 ^{Bb}	74.7±11.5 ^{Bb}	84.9±12.8 ^{Cc}

Different superscript capital letters represent a statistically significant difference between different materials at the same concentration ($p < 0.05$).

Discussion

An ideal endodontic filling material for primary teeth must fulfill a number of requirements that makes it convenient to use. One of them would be the low cytotoxicity to the periapical tissues, so that when extruded from the apex the paste does not trigger an inflammatory reaction affecting the eruption of the permanent tooth.¹⁶

Cytotoxicity is the intrinsic capacity of a material to promote metabolic alterations in cells, which may culminate in cell death.¹⁷ *In vitro* tests allow the evaluation of the cytotoxic potential of different materials and have been replacing *in vivo* tests, mainly due to their reduced cost, high reproducibility and easy performance.^{17,18} Cell quantification performed using the MTT colorimetric method evaluate the mitochondrial activity of living cells that convert MTT into blue crystals of soluble formazan and, consequently, allows the measuring of the cell survival in the media.¹⁹

In this study, the pastes used for endodontic therapy in primary teeth Calen PMCC, Feapex and CTZ were subjected to the cytotoxicity test of cell viability. This test was applied to Human Osteoblasts-like of the SAOS-2 strain. These cells, derived from osteosarcoma, have several osteoblastic characteristics and could be used as a line similar to human osteoblasts and as a source of bone-related molecules.²⁰

In addition to their worldwide availability, some of the advantages that highly indicate the use of SAOS-2 strain are that they have well-documented characterization data, the possibility of obtaining large amounts of cells in a short period of time and the fact that SAOS-2 cells can be totally differentiated as the way that osteoblastic cells naturally do.²¹

The null hypothesis was rejected in the present study, since the cell viability results demonstrated that the samples submitted to Feapex paste presented greater cell viability than the other tested pastes (Calen PMCC and CTZ) ($p < 0.05$). These findings are in accordance with previous studies that showed that iodoform-based materials are easily absorbed and do not cause foreign body reactions, leading to a high cell survival rate.²²⁻²⁴

In this study, the cytotoxicity of the CTZ paste was higher than the cytotoxicity of the other materials tested, which can be justified since any material that includes eugenol in its formulation can trigger a severe tissue reaction due to the harmful effects of this component on the cytoplasmic membrane and cellular respiratory depression.^{25, 26} On the other hand, the CTZ paste at the lowest concentrations (1:1 and 1:2) showed similar cytotoxicity to the Calen PMCC

paste, corroborating with previous findings that showed that the Calcium Hydroxide-based paste is not less cytotoxic than others usual filling materials.²⁷ Their initially high degree of toxicity seems to decrease significantly with the time of exposure and it ends up presenting characteristics of biocompatibility without a significant inflammatory response.³

In view of the results presented, it is worth mentioning that other studies must be carried out to continuing assess the effectiveness of the tested filling materials, such as the evaluation of their cytotoxicity effects in different periods of exposure. Dental professionals should be aware about the possible detrimental effects related to the use of eugenol and calcium hydroxide-based pastes in pediatric endodontic treatment.

Conclusions

According to the results of the present study, it can be concluded that the Feapex endodontic paste seems to be the better option for use in endodontic treatment of primary teeth among the analyzed pastes, since it presented lower cytotoxicity than Calen PMCC and CTZ pastes.

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