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Hot air stream reduces cytotoxicity of light-cured calcium hydroxide based cements

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Abstract

Background: The light-cured calcium hydroxide based cements have incomplete polymerization and unconverted monomers can cause pulp cell damage. The aim of this study was to evaluate the influence of a warm and hot air stream on the cytotoxicity of light-cured calcium hydroxide based cements.

Material and Methods: The materials Dycal (conventional cement), Biocal, Hidrox-Cal, and Ultra-Blend Plus (light-cured calcium hydroxide cements) were submitted to cytotoxicity analysis after polymerization, without vs. with previous heat treatment with a warm (37°C) and a hot (60°C) air stream. Following polymerization, cements were maintained in culture medium for 24 hours and 7 days, and subjected to the MTT test. Data were analyzed using analysis of variance (ANOVA) followed by post-hoc Student-Newman-Keuls ($p < 0.05$).

Results: The results indicated significant differences between the materials according to their composition, i.e., light-cured cements treated with a jet of warm air showed similar cytotoxicity levels to those observed for conventional cement, suggesting that they may be considered alternatives in cases requiring pulp-capping treatment.

Conclusions: Application of a hot air stream reduced cytotoxicity of materials tested.

Key words: Dental pulp capping, dental cements, calcium hydroxide, cell survival.

Introduction

First introduced in Germany in 1920, calcium hydroxide has been the biomaterial most extensively studied over the years, with favorable clinical results (1). Calcium hydroxide may be used directly on pulp tissues or indirectly as pulp-capping material (2). It has a long history of clinical success and continues to be the gold standard when the goal is to protect the dentin-pulp complex (3,4). The main advantage of calcium hydroxide is its alkaline pH, as it creates an optimal setting for the stimulation of alkaline phosphatase, essential to the formation of mineralized barrier (5).

From a different standpoint, calcium hydroxide presents a low modulus of elasticity and low resistance to compression, which restrict its use in thin layers, in specific areas (6). The limited mechanical properties of chemically activated calcium hydroxide, combined with the requirement to accommodate the material in regions with the eminence of pulp exposure, has resulted in the development of light-curable calcium hydroxide based cements, composed of methacrylate monomers, with the following advantages: increased resistance, low solubility in acid settings, controlled working time, and achievement of optimal mechanical properties immediately after the light-curing process (7).

However, when resin materials are applied to deep cavities, diffusion of unpolymerized monomers into pulp tissues may occur through the dentinal tubules (8-10) and cause toxic effects (11-13). This situation can become more severe when the monomers come into direct contact with the dental pulp (14). Therefore, adequate conversion of monomers into polymers is essential to maximize the physical properties and clinical performance of light-curable calcium hydroxide based cements, as well as to reduce their cytotoxicity (15). Among several exist-

ing strategies to enhance polymerization rates, the use of high temperatures either before or during polymerization stands out (16-18). Several authors have demonstrated higher polymerization rates and therefore superior physical (16,19) and biological (20) properties of resin materials after the use of light-curing methods employing heat.

A fundamental feature for these materials is biocompatibility (21) and the first step in assessing biocompatibility is through cytotoxicity analysis based on cellular behavior and viability (22). Methacrylate-based dental materials are known to present a high level of cytotoxicity, and are therefore likely to penetrate the pulp and induce cytotoxic effects (23). Therefore, the aim of this study was to evaluate the influence of a warm and hot air stream on the cytotoxicity of light-cured calcium hydroxide cements.

Material and Methods

-Sample preparation

A conventional cement - Dycal (Dentsply - Milford, DE, USA) and three light-cured calcium hydroxide based cements: Biocal (Biodinâmica, Ibiporã, PR, Brazil), Hidrox-Cal (Maquira, Maringá, PR, Brazil), Ultra-Blend Plus (Ultradent Products Inc., South Jordan, UT, USA) were used in this *in vitro* study (Table 1). Conventional cement was mixed by 10s before heat treatment. All cements were treated in three different forms: 1) no heat treatment; 2) treatment with a jet of warm air (37°C); 3) treatment with a jet of hot air (60°C). The jet air application lasted 5 seconds, distant 10 cm from the slide. All light-curable specimens were light-cured for 20 seconds using a VALO cordless light-emitting diode (LED) curing unit (Ultradent, Salt Lake City, UT, USA). The samples measuring 9 mm diameter x 1 mm thickness were prepared and immediately sterilized with ethylene oxide (Esterilplus, Porto Alegre, RS, Brazil), (Table 1).

Table 1: Calcium hydroxide based cement used, composition, batch, and manufacturer.

Cement	Composition*	Manufacturer (batch number)
Dycal	Base paste: 1,3-butylene glycol disalicylate, zinc oxide, calcium phosphate, calcium tungstate, iron oxide pigments Catalyst paste: calcium hydroxide, N-ethyl-o/p-toluene sulfonamide, zinc oxide, titanium dioxide, zinc stearate, iron oxide pigments (dentine shade only).	Dentsply (2848551)
BioCal	Calcium, ethylene urethane dimethacrylate, inorganic fillers, barium sulfate, photoactivator, and pigments	Biodinâmica (1A118D03)
Hidrox-Cal	Calcium hydroxide (7.5%), urethane dimethacrylate, tetraethylene glycol dimethacrylate, butylhydroxytoluene, camphorquinone, chivacure EPD, glass filler 1.0 µm, yellow iron oxide, red iron oxide, titanium dioxide	Maquira (028316)
Ultra-Blend Plus	Calcium hydroxide, calcium hydroxyapatite, urethane dimethacrylate	Ultradent (D02B6)

* As informed by the manufacturer.

-Cell culture

The cells used in this study were NIH/3T3 mouse fibroblasts (ATCC®-American Type Culture Collection-TCC, Old Town, MD, USA) cultured in Dulbecco's Modified Eagle Media (DMEM; Invitrogen®, Carlsbad, CA, USA). This medium was supplemented with 10% of fetal bovine serum, 100 U/mL of penicillin (Gibco), 100 U/mL of streptomycin (Gibco), and 100 µg/mL of gentamycin (Gibco). Cells were kept in a humidified incubator at a temperature of 37°C and 5% of CO₂.

-Culture medium

Immediately after the mixing/curing process, specimens from the four groups were immersed in the DMEM medium. The specimen surface area to medium volume ratio was 3 cm²/mL, according to ISO 10993-12 (24). Surface area was calculated based on the total dimensions of the specimen, disregarding porosity. Extracts were tested for cell viability after remaining 24 hours and 7 days in the incubator.

-Cytotoxicity assay

The MTT method (Sigma-Aldrich, St. Louis, MO, USA) was used to assess cytotoxicity using the same parameters described elsewhere³¹. Briefly, at each treatment time (24 hours and 7 days), the culture medium was removed and 10% of an MTT solution (5 mg/mL) in phosphate buffer solution was added to each well. Subsequently, cultures were incubated at 37°C, protected from light, until the presence of blue-to-purple formazan crystals was observed. For the solubilization of formazan crystals, 100 µL of dimethyl sulfoxide (DMSO) was added to each well, and absorbance was measured at 570 nm wavelength using a spectrophotometer and an ELISA microplate reader (Benchmark Microplate Reader, Bio-Rad Inc., Hercules, CA, USA). The percentage of viable cells was calculated and compared to the results obtained with the negative control (cells cultured in DMEM). The assay was validated using a positive toxicity control (cells treated with 2% sodium hypochlorite).

-Statistical analysis

The cytotoxicity of calcium hydroxide based cements without previous heat treatment vs. those treated with a warm and a hot air stream were compared in terms

of cell viability rates in NIH/3T3 mouse fibroblast cultures using three-way analysis of variance (ANOVA) followed by the post-hoc Student-Newman-Keuls test. Examiners were blinded to group allocation. Results were expressed as mean and standard deviation. Significance was set at 5%.

Results

Table 2 shows the cell viability results obtained for the four calcium hydroxide based materials. Application of a warm or hot air stream did not influence cell viability in the conventional calcium hydroxide cement Dycal, which presented higher viability rates than resin-modified materials. Conversely, in all resin-based calcium hydroxide materials, the hot air stream enhanced cell viability at both 24 hours and 7 days. All tested materials presented lower rates of cell viability at 7 days compared to 24 hours.

Discussion

Light-curing pulp-capping materials were developed with the goal of simplifying the operating technique by facilitating material application. Due to their resin base, these materials are associated with increased mechanical resistance (4) and improved marginal seal as a result of lower dissolution rates (25). The present study showed that treatment of these materials with a jet of warm air just before their application resulted in cytotoxicity rates similar to those reported for chemically activated cements, most widely used at present. However, studies have shown that these cements, even when used for pulp capping, present a certain degree of cytotoxicity – a fact that becomes extremely relevant, as these cements are often used very close to dental pulp tissues.

The cytotoxicity associated with light-curing calcium hydroxide based cements and dentin adhesives is possibly a result of residual monomers (8). The monomer to polymer conversion rate for this type of material (dimethacrylate-based) is approximately 70%. Taking into consideration the proportion of monomers not completely converted and their possible diffusion into pulp tissues, it has been estimated that around 9% may leach

Table 2: Mean ± standard deviation of cell viability rates (%) obtained at 24 hours and 7 days in light-cured and mixed calcium hydroxide based materials without and with previous heat treatment.

Cement	24 hours			7 days		
	No heating	37°C	60°C	No heating	37°C	60°C
Dycal	33.56±0.99A	31.80±0.75A	31.32±0.48A	15.11±2.99C	17.57±0.71C	16.89±0.18C
BioCal	15.62±0.11C	13.87±0.39E	33.11±0.66A	17.97±1.21C	16.53±0.45C	17.38±0.44C
HidroX-Cal	10.32±1.10B	11.12±0.72B	30.59±1.38A	5.96±0.64D	6.09±0.36D	17.72±0.44C
Ultra-Blend	10.28±0.38B	9.51±0.60B	32.45±1.32A	5.32±0.90D	6.89±0.64D	16.02±0.58C

Different letters indicate statistical differences ($p < 0.05$).

when in contact with fluids (4). Therefore, the use of heat treatment before application of these materials has been investigated in an attempt to increase conversion rates and reduce the number of free monomers, and consequently the cytotoxic potential of the materials (20). The higher polymerization rates obtained as a result of heat treatment are explained by the increased mobility of monomers when heated (19).

In this study, treatment with a hot air stream (60°C) yielded significantly increased cell viability rates in methacrylate resin-based calcium hydroxide cements, both at 24 hours and at 7 days in comparison with the specimens that were not subjected to heating and with those treated with a jet of warm rather than hot air (37°C). In addition, the cell viability results observed in the two latter groups (no heating and heating to 37°C) was not different than the results observed for Dycal. Conventional cements have salicylate resin in their composition, and chemical curing occurs through chelation of calcium ions by the salicylate group; therefore, cell viability results in our conventional cement Dycal were not influenced by heat treatment, but it does influence monomer conversion in methacrylate resin-based cements. This explains the results obtained in the present study with BioCal, Hidrox-Cal, and Ultra-Blend Plus, which contain a urethane dimethacrylate-based matrix in their composition. Cell viability results in the present study were similar to those found by the same authors in a previous study designed to assess self-adhesive resin cements (21). Also in that study, heat treatment to 60°C increased cell viability of fibroblasts, but viability levels reduced after 7 days. It is likely that a longer extraction time causes a larger number of unpolymerized monomers to leach from the polymerized resin matrix and provoke cell non-viability. Still, the level of cytotoxicity observed seems to be compatible with that observed in other materials with adequate clinical performance (26).

As already mentioned, the increased cell viability in dental materials is probably associated with an increased monomer-to-polymer conversion rate (20). However, in the present study, conversion rates were not measured; rather, cell viability essays were used, and viability was considered as a proxy for the effectiveness of the polymerization process. Other factors that could interfere with the biocompatibility of pulp-capping materials, e.g., release of calcium ions, alkalizing potential, solubility and antibacterial activity (27-29) should be explored in future studies.

In general, heat treatment through application of a hot air stream at 60°C before light-curing calcium hydroxide based cements reduced the cytotoxicity of materials. The adoption of this strategy as routine is justified in treatments that require pulp-capping, especially as it does not require an extra clinical step.

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Conflict of interest

The authors have declared that no conflict of interest exist.