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## Human Periodontal Ligament Cells Response to Commercially Available Calcium Hydroxide Pastes

### Research Article

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### Abstract

Several studies have shown that calcium hydroxide based medicaments have a cytotoxic effect on human cells. The purpose of this study was to evaluate the cytotoxicity of several calcium hydroxide products on periodontal ligament (PDL) cells. Calcium hydroxide powder (Avantor Performance Materials Inc.), Calasept® (Nordiska Dental AB), Metapaste® (Meta Biomed Co., Ltd.), Vitapex® (Neo Dental International Inc.), Ultracal® (Ultradent Products, Inc.), and Pulpdent® (Pulpdent Corporation) products were tested. PDL cells were exposed to various concentrations of calcium hydroxide from each product (1.0, 0.5, 0.25, and 0.125 mg/mL). Cell viability was measured after 24 h and 48 h by Cell Proliferation Assay. All materials tested had a more toxic effect on PDL cells after 48 h. At 24 and 48 h, Metapaste® was the most toxic regardless of concentrations used. Products with a 1.0 and 0.5 mg/mL concentration had statistically significant more cytotoxic effects when compared to the negative control. Pure calcium hydroxide and Calasept® induced 35% cell death at a 1 mg/mL concentration and 15-20% cell death at 0.5, 0.25, and 0.125 mg/mL after 24 h. Pulpdent® and Ultracal® induced 30-35% cell death at a 1 mg/mL concentration and its effect diminished at 0.25 and 0.125 mg/mL at 24 h. The Vitapex® preparation induced 20% PDL cell death at 24 h regardless of the concentration and was the least toxic significantly at 1 mg/mL compared to other brands, except Pulpdent®, at the same concentration at 24 h. All calcium hydroxide products showed evidence of cytotoxicity on PDL cells, with Metapaste being the most cytotoxic. The cytotoxicity was related to concentration and exposure time. Pulpdent® and Ultracal® had excellent biocompatibility at lower concentrations.

**Key words:** Cytotoxicity, Calcium hydroxide, Human cells, Fibroblasts.

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### Introduction

Calcium hydroxide is the de facto standard intracanal medicament for root canal disinfection and the promotion of chronic apical periodontitis healing [1-3]. At first, clinicians were mixing calcium hydroxide with water to produce an aqueous paste. Then, several studies tested different vehicles and formulations to improve handling properties, antibacterial actions, physical behavior, and radiopacity. Currently, there are several commercial preparations available [4]. The biological consequences of different mixing vehicles are not fully understood.

The basic mechanism action of calcium hydroxide is based on the dissociation of  $\text{Ca}^{2+}$  and  $\text{OH}^-$  ions. The outcome of such dissociation results in an elevated pH. Calcium hydroxide has been shown to have antimicrobial activity when used clinically or with in vitro models. Intracanal calcium hydroxide rendered infected root canals free of cultivable bacteria after seven days [2,5]. It has been shown that 92.5% of canals become bacteria free using the same protocol [6]. However, others have shown that calcium hydroxide application between appointments did not have any effect on disinfecting canals or treatment outcome [7,8]. Furthermore, a more recent systematic review of eight clinical trials indicated that calcium hydroxide was ineffective in eliminating bacteria from infected root canals [9]. The differences in these studies may be attributed to the different calcium preparations used. It was suggested that certain vehicles may decrease the antimicrobial effectiveness of calcium hydroxide [10]. Nevertheless, studies are lacking to compare antimicrobial activities of different calcium hydroxide preparations.

It is well known that calcium hydroxide is cytotoxic. Several *in vitro/ex vivo* and animal studies were conducted to test individual products. Intracanal medicaments are usually confined within the root canal system. Yet, through extrusion, calcium hydroxide may invade the periapical tissues. Some authors have advocated such practices to improve the outcome of teeth with periapical lesions and thus change the environment of chronically inflamed tissue and epithelial cystic linings [11]. But, case reports have alluded to bone necrosis and neurotoxicity as a result of this approach [12]. The lack of periapical healing and more complications have been reported after the extrusion of calcium hydroxide [13]. Others have shown that sealer containing calcium hydroxide had a toxic effect on rat sciatic nerve, yet it appeared this effect diminished

after 60 minutes [14,15]. Direct contact of calcium hydroxide with nerve tissue for 30 minutes caused irreversible damage [15]. Periodontal ligament cells (PDL cells) play a crucial role in the regeneration and repair process of chronic apical periodontitis [16]. Due to the fact that any intracanal medicaments used will come in close contact or accidentally injected outside the root canal system, it should not prevent nor interfere the healing process or be toxic to PDL cells [17]. Therefore, the present study was designed to evaluate the cytotoxicity of several calcium hydroxide products on primary PDL cells.

## Methods

### Calcium hydroxide brands used

Six brands of commercially available dental calcium hydroxide medicaments were purchased. Calcium hydroxide powder (Avantor Performance Materials Inc.), Calasept® (Nordiska Dental AB), Metapaste® (Meta Biomed Co., Ltd.), Vitapex® (Neo Dental International Inc.), Ultracal® (Ultradent Products, Inc.), and Pulpdent® (Pulpdent Corporation) products were selected. All material safety data sheet (MSDS) were obtained from the manufacturer's websites.

### Sample preparation, pH readings and cell proliferation assay

All calcium hydroxide products were mixed with culture media (Dulbecco's modified Eagle's medium culture medium (Life Technologies, NY) supplemented with antibiotics and 10% fetal bovine serum (FBS). Each product was serially diluted at ratios of 1.0, 0.5, 0.25, and 0.125 mg/mL. The pH of each preparation was measured using Accumet basic pH meter (Waltham, MA).

PDL cells were obtained from ScienCell (Carlsbad, CA) and grown in complete Dulbecco's modified Eagle's culture medium (Life Technologies, NY) supplemented with antibiotics and 10% FBS at 37°C in a humidified 5% CO<sub>2</sub> atmosphere incubator. Cells between the 4th and 6th passages were used. A total of 15 x 10<sup>3</sup> PDL cells were plated in culture medium onto 96-well

plates and grown overnight to allow adherence to the surface. For the experimental groups, different calcium hydroxide brands of various concentrations were added to each well. PDL cells treated with 6% sodium hypochlorite served as the positive control. PDL cells kept in fresh medium served as the negative control. After 24 and 48 h, PDL cells proliferation was evaluated by CellTiter96 AQueous Non-Radioactive Cell Proliferation Assay (Madison, WI), composed of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS], according to the manufacturer's instructions. The optical density (OD) values for each well were measured spectrophotometrically at 490 nm.

### Data analysis

The data obtained from the MTS proliferation assay were recorded and analyzed by a statistician. The difference in means between the calcium hydroxide brands, time, and concentrations was made by using a three factor analysis of variance with a means separation test using SAS 9.4.

## Results

### MSDS data and pH measurement

Relevant clinical data on the material safety data sheet of each product was summarized in table 1. The highest concentration calcium hydroxide was reported in the pure USP brand, followed by Pulpdent® which reported 42%. Other brands ranged from 30-35%. Calasept®, Ultracal®, and Pulpdent® are aqueous preparations. The use of propyleneglycol makes Metapaste® a viscous preparation and reports an unspecified calcium hydroxide percentage. Vitapex® contains 40% idoform and silicone oil as a carrier.

The pH readings of all materials tested with different concentrations were shown in table 2. Pure calcium hydroxide had the highest pH reading. Metapaste® and Vitapex® were the lowest. The pH readings were lower as the concentration of materials tested decreased.

**Table 1. Relevant clinical data on the material safety data (MSDS) sheet of each product.**

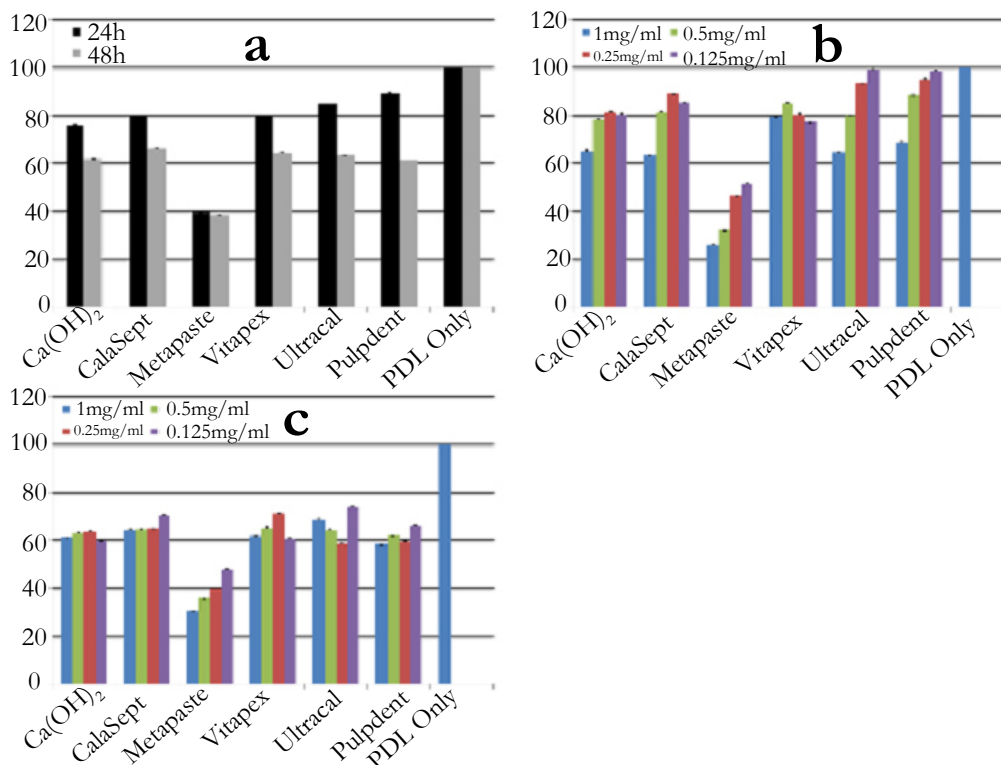
Brand	Manufacturer	Ca(OH) <sub>2</sub>	Radio-pacifiers	Vehicle	Hazardous Materials	Additional Materials
Ca(OH) <sub>2</sub>	Avantor Performance Materials Saucon Valley Plaza 3477 Corporate Pkwy Suite #200 Center Valley, PA 18034	Pure powder	NA	Sterile H <sub>2</sub> O (aqueous)	Calcium Hydroxide Corrosive Causes burning of skin and eyes	NA
Calasept®	Nordiska Dental AB Box 1082 S-262 21 Angelholm Sweden	>35% (not greater than 56%)	Barium Sulphate >5%	H <sub>2</sub> O (aqueous)	Calcium Hydroxide Corrosive Causes burning of skin and eyes	Ringer solution >30%. CaCl 8mg (NaCl 0.35mg) (KCl 8mg) (NaHCO <sub>3</sub> 4mg)
Metapaste®	Meta Biomed Co., Ltd. 414-12 Mochung-Dong, Heungdeok-gu, Cheongju City, Chungbuk, 361-808, Korea	Unspecified	Barium Sulphate	propyleneglycol(viscous)	Calcium Hydroxide Corrosive Causes burning of skin and eyes	NA

Vitapex®	Neo Dental International Inc. 2505 South 320th Street, Suite #250 Federal Way, Wa98003	30%	Iodoform 40%	Silicone oil(Oily)	Calcium Hydroxide Corrosive Causes burning of skin and eyes	Unknown 6.9%
Ultracal XS®	Ultradent Products, Inc. 505 W. 10200 S. South Jourdan, UT 84095	35%	Barium Sulphate 20%	proprietary(aqueous)	Calcium Hydroxide Corrosive Causes burning of skin and eyes	NA
Pulpdent®	Pulpdent Corporation 80 Oakland Street 24 Watertown, MA 02472 USA	42%	Barium Sulphate	Methylcellulose paste(aqueous)	Calcium Hydroxide Corrosive Causes burning of skin and eyes	NA

**Table 2.**The pH readings of all materials tested with different concentrations.

Product	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL
Ca(OH) <sub>2</sub>	9.39	8.7	8.34	8.05
Calasept®	8.96	8.46	8.22	8.08
Metapaste®	8.43	8.32	8.07	7.93
Vitapex®	8.42	8.18	8.06	7.92
Ultracal XS®	8.88	8.27	8.07	7.93
Pulpdent®	9	8.42	8.09	7.94

**Figure 1.** Different commercial calcium hydroxide preparations have different cytotoxic effects on PDL cells. Approximately 15 x 10<sup>3</sup> PDL cells were plated in culture medium onto 96-well plates and treated with different calcium hydroxide brands of various concentrations. (a) mean ± standard error representing percentage of PDL survival obtained after 24 or 48 h from exposure to each product (pool of data obtained from all concentrations used). (b-c) mean ± standard error representing percentage of PDL survival obtained after 24 or 48 h from exposure to each product of various concentrations. Bar graphs represent mean of relative levels of 5 samples and 3 independent experiments.



**PDL cells Proliferation**

All materials tested had a more toxic effect on PDL cells after 48 h (Fig. 1a). At 24 and 48 h, Metapaste® was the most toxic regardless of the concentration used. Interestingly enough, the Metapaste® at 0.125 mg/mL was significantly more toxic than other brands at 1 mg/mL at the 24 and 48 h intervals (Fig. 1b&c). Pure

calcium hydroxide mixed with water and Calasept® induced 35% cell death at a 1 mg/mL concentration and 15-20% cell death at 0.5, 0.25, and 0.125 mg/mL after 24 h (Fig. 1b). Pulpdent® and Ultracal® appeared to have similar outcomes on PDL cell death. Both induced 30-35% cell death at a 1 mg/mL concentration and its effect diminished at 0.25 and 0.125 mg/mL at 24 h (Fig. 1b). The Vitapex® preparation induced 20% PDL cell death at 24 h regardless of the concentration. Furthermore, Vitapex® was the

least toxic significantly at 1 mg/mL compared to other brands, except Pulpdent®, at the same concentration at 24 h (Fig. 1b). At 48 h, all brands, except Metapaste®, had very similar toxic effect (Fig. 1c). Sodium hypochlorite at 6% induced 100% cell death at 24 and 48 h (data not shown).

## Discussion

The antimicrobial effect of calcium hydroxide has been studied extensively with different outcomes. Early studies reported high success with the use of calcium hydroxide [2]. However, a more recent systematic review of eight clinical trials indicated that calcium hydroxide is ineffective in eliminating bacteria from infected root canals [18]. The early studies used pure calcium hydroxide mixed in-office. Currently, commercial preparations are convenient to use. The antimicrobial effect of calcium hydroxide may be compromised when using different delivery vehicles [10]. The present study, for the first time, compares the cytotoxicity of several commercial preparations on PDL cells. PDL cells are crucial during the early infection as well as the resolution stage of infection at the root surfaces [16]. Therefore, it would be prudent to use a product that is least toxic to the PDL cells. Moreover, it would be interesting to evaluate the anti-microbial effect of the least toxic commercial calcium hydroxide product in the future.

As endodontists, the intention is to keep any intracanal medications within the root canal system. Yet, calcium hydroxide may extrude beyond the apical foramen. Some authors have advocated such practices to improve the outcome of teeth with periapical lesions to change the environment of chronically inflamed tissue and epithelial cystic linings [11]. Furthermore, emerging evidence indicates that calcium hydroxide has an application in endodontic regeneration [19-21]. Calcium hydroxide significantly increased the proliferation of stem cells of apical papilla [22]. The present study demonstrated that different preparations have different effects on PDL cell survival, regardless of concentration. Metapaste® was the most toxic and had lower pH readings compared to others. It is considered a viscous preparation, in which propyleneglycol is used as the vehicle and barium sulphate for radiopacity. The results presented suggest that the toxic effect was not only due to calcium hydroxide, but from other component(s), especially those in Metapaste®.

It appears that Ultracal® and Pulpdent® have the least toxic effect at 0.25 and 0.125 mg/mL. Both brands are considered aqueous preparations that facilitate the dissociation reaction required for calcium hydroxide to be effective [23]. It should be noted that the pH of these concentrations is close to 8. With emerging evidence of extra radicular microorganisms and biofilms, it would be advantageous to use a preparation with the least toxic effect on the host.

In conclusion, different commercial calcium hydroxide preparations have different cytotoxic effects on PDL cells, with Pulpdent® and Ultracal® exhibiting excellent biocompatibility at lower concentrations.

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est.

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