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Light-curing of calcium hydroxide-based liners: pH analysis and calcium ion release

Fotoativação de forradores a base de hidróxido de cálcio: análise de pH e liberação de íons cálcio

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ABSTRACT

Objective: Compare the pH values and calcium ion release of calcium hydroxide-based liner materials before and after light-curing. **Material and Methods:** The materials evaluated were: hydrox-cal white (HW), hydrox-cal dentin (HD), Biocal (BC) and UltraBlend Plus (UB). 120 samples of the liner materials were inserted into a PVC tube (n=15). The samples from HW+A, HD+A, BC+A and UB+A were subjected to photoactivation. The other groups HW+N, HD+N, BC+N and UB+N were only inserted in a glass tube with deionized water. The pH was measured 24 hours and 14 days after the inclusion of the samples with the aid of a pH meter. The calcium release was analyzed with the aid of an atomic absorption spectophotometer at 24h and 14 days. The results were submitted to the Shapiro-Wilk test, followed by ANOVA and Tukey test (p=0.05). **Results**: In 24h, the groups that were not light cured showed the highest pH values (p<0.05). In 14 days, BC+N and BC+A demonstrated the lowest pH values. The groups that were not light cured also showed higher calcium release values in 24h and 14 days (p<0.05). **Conclusion**: Photoactivation of calcium hydroxide-based liner materials negatively interferes with calcium ion release, as well as with pH.

KEYWORDS

Acidification; Alkalinization; Calcium hydroxide; Hydrogen-ion concentration; Light-curing.

RESUMO

Objetivo: Comparar os valores de pH e liberação de íons cálcio de materiais forradores à base de hidróxido de cálcio antes e depois da fotopolimerização. **Material e métodos:** Os materiais avaliados foram: Hidrox-cal branco (HW), Hidrox-cal dentina (HD), Biocal (BC) e UltraBlend Plus (UB). 120 amostras dos materiais de revestimento foram inseridas em um tubo de PVC (n=15). As amostras de HW +A, HD+A, BC+A e UB+A foram submetidas à fotoativação. Os demais grupos HW +N, HD+N, BC+N e UB+N foram inseridos apenas em um tubo de vidro com água deionizada. O pH foi medido 24 horas e 14 dias após a inclusão das amostras com o auxílio de um medidor de pH. A liberação de cálcio foi analisada com o auxílio de um espectrofotômetro de absorção atômica em 24h e 14 dias. Os resultados foram submetidos ao teste de Shapiro-Wilk, seguido de ANOVA e teste de Tukey (p=0,05). **Resultados:** Em 24h, os grupos não fotopolimerizados apresentaram os maiores valores de pH (p<0,05). Em 14 dias, BC+N e BC+A apresentaram os menores valores de pH. Os grupos não fotopolimerizados também apresentaram maiores valores de liberação de cálcio em 24h e 14 dias (p<0,05). **Conclusão:** A fotoativação de materiais de revestimento à base de hidróxido de cálcio interfere negativamente na liberação de íons cálcio e no pH.

PALAVRAS-CHAVE

Acidificação; Alcalinização; Concentração de íons de hidrogênio; Fotopolimerização; Hidróxido de cálcio.

INTRODUCTION

Calcium hydroxide was introduced in dentistry in 1920 and is highly recommended as a protective agent for the dentin-pulp complex [1]. The material has high solubility and slow release of calcium and hydroxyl ions [2] when in contact with humid environment [1]. The presence of calcium ions in dentin promotes remineralization, while the hydroxyl ion inhibits the action of microorganisms and alkalizes the pH of the region [3], thus promoting the formation of hard tissue [1].

Several materials are proposed in the literature as protective agents of the dentin-pulp complex. The objective of using these materials is to prevent pulpal exposure in cases of extensive and deep caries, besides favoring dental remineralization by the formation of restorative dentin in the region [4]. Calcium hydroxide is still one of the main materials of choice for this procedure due to alkalinization, biocompatibility and remineralization, which provides formation of tertiary dentin [4,5].

However, their high solubility, poor mechanical properties, and lack of adhesion to dentinal tissue make them unviable. As a solution to the problem, some calcium hydroxide-based materials that allow photoactivation have appeared in the market [5,6]. The addition of polymerizable methacrylates allowed their physical properties, chemical stability and solubility to be improved [7-9], making their clinical use more favorable and practical.

Although photoactivation replaces the deficiencies that calcium hydroxide cements had, its use may prevent or reduce the release of calcium and hydroxyl ions into the tooth tissue, besides not achieving a good pH stability, thus affecting dentin remineralization [3]. However, there are controversies about this change in pH and ion release using light-cured calcium hydroxide-based materials [6]. Therefore, it is of interest to evaluate whether light-curing interferes with the beneficial properties of calcium hydroxide-based materials. Therefore, it is necessary to compare the pH values and the presence of calcium ions before and after light curing.

The aim of this study was to evaluate the hydrogen potencial and calcium release of cavity luting cements containing calcium hydroxide (Hydrox-cal white, Hydrox-cal dentin, Biocal and Ultra-Blend Plus) before and after photoactivation, at 24 hours and 14 days, by measuring pH with a pH meter and by atomic absorption spectroscopy to evaluate calcium ions. The null hypotheses (H0) there were no differences in relation to pH and calcium release in the calcium hydroxide cements with or no photoactivation.

MATERIAL AND METHODS

The analyses were performed by a single operator who did not know which groups were being evaluated. Table I shows the materials used, manufactures and chemical composition.

Evaluated groups

- HW+N (Hydrox-cal white, not photoactivated): the material was directly inserted into the polyethylene tube and immediately immersed in distilled water; - HW+A (Hydrox-cal white, photoactivated): After the material was inserted in the polyethylene tubes, the set underwent photoactivation using a LED unit (Valo; South Jordan, UT, USA) at potency of 1200mW/cm^2, positioned 5 mm apart for 20

Table I - Liner materials, manufacturer and chemical composition

Materials	Manufacturer	Chemical compositiom
Hidrox-cal white	Maquira, (Maringá, PR, BR)	Calcium hydroxide (7.5%), UDMA, Tetraethylene glycol dimethacrylate, Subtle Hydroxy Toluene, Camphorquinone, Chivacure EPD, 1.0µm glass filler
Hidrox-cal dentin	Maquira, (Maringá, PR, BR)	Calcium Hydroxide (7.5%), UDMA, Tetraethylene Glycoldimethacrylate, Subtle Hydroxy Toluene, Camphorquinone, Chivacure EPD, 1.0 µm Glass Filler, Yellow Iron Oxide, Red Iron Oxide, Titanium Dioxide
Biocal	Biodinâmica (Ibiporã, PR, BR)	Calcium hydroxide, UDMA, inorganic particles, barium sulfate, photoactivator and pigments
Ultra-Blend Plus	Ultradent (South Jordan, UT, USA)	UDMA, calcium hydroxide

seconds. Immediately, the specimens were immersed in distilled water;

- HD+N (Hydrox-cal dentin, not light cured): similar to HW +N, but Hydrox-cal dentin was used;
- HD+A (Hydrox-cal dentin light cured): similar to HW+A, but Hydrox-cal dentin was used;
- BC+N (Biocal, not light cured): similar to HW+N, but Biocal was used;
- BC+A (Biocal, light cured): similar to HW+A, but Biocal was used;
- UB+N (Ultra-blend Plus, not light cured): similar to HW+N, but Ultra-blend Plus was used;
- UB+A (Ultra-blend Plus, light cured): similar to HW+A, but Ultra-blend Plus was used.

pH analysis

Fifteen specimens from each group were prepared for the study, totaling 120 individualized samples. The materials, described in Table I, were inserted in polyethylene tubes (10 mm length x 1 mm internal diameter) compacted at the ends, with a spatula 24, to avoid lateral extrusion of the materials. To standardize the volume contained in the specimens, the set was weighed on an analytical balance, with precision of 0.0001g (ATY224; Shimadzu, São Paulo, SP, BR), and maintained at a weight of 10 + 0.1 mg. After insertion and standardization of the volume of materials in polyethylene tubes, HW+A, HD+A, BC+A and UB+A were subjected to photoactivation. Immediately after, all specimens were individually immersed in glass vials with 10 mL of distilled deionized water with controlled pH (pH=7), sealed with a plastic cap and kept at rest at a constant temperature of 37ºC. After 24 hours, the pH of the distilled water was measured with a pH meter (Q-400; Quimis Instrument, São Paulo, SP, BR), previously calibrated with buffered solutions (pH 4.0 and 7.0), as described by Duarte et al. (2007) [9].

Calcium release analysis

After pH measurement, the solution in which the specimens were immersed was analyzed to quantify the presence of calcium released by the materials. For this, an atomic absorption spectrophotometer (Spectra 55B - Varian, Inc., Palo Alto, CA, USA) was used.

Initially, lanthanum oxide was added to all samples in a proportion of 1% in relation to the volume of the initial content, to avoid the interference of phosphate ions on the analysis. After calibration of the spectrophotometer with standard solutions containing calcium at the values of 0, 1, 2, 3, 4 and 5 ppm, the measurement was performed.

After the pH and calcium release measurements were completed within 24 hours, the specimens were again immersed in 10 mL of distilled water. The set was stored and kept in the same conditions as previously described. After 14 days, a new measurement of pH and calcium release in the solution was obtained.

Statistical analysis

The results obtained from the pH and calcium release analyses were submitted to the Shapiro-Wilk tetes to evaluate the homoscedasticity of the data. Then, the values were analyzed by ANOVA and Tukey ($\alpha=0.05$) tests.

RESULTS

pH analysis

After 24 h of immersion in distilled water, HW+N, HD+N, and UB+N provided the highest pH values ($P<0.05$). BC+A provided the lowest pH value ($P<0.05$). On the other hand, HW+A and HD+A provided higher pH value than BC+N ($P<0.05$). There were no differences between HW+N, HD+N and UB+N or between HW+A, HD+A and UB+N ($P>0.05$).

After 14 days, BC+N and BC+A provided the lowest pH values ($P<0.05$) but were similar to each other ($P>0.05$). There was no difference among the other groups ($P>0.05$).

Table II shows the arithmetic mean and standard deviation of the pH values at 24 hours and 14 days of immersion in distilled water as a function of photoactivation of calcium hydroxide-containing pulp protection agents.

Calcium release analysis

After 24, HW+N, HD+N, and UB+N provided the highest calcium release values ($P<0.05$), but similar to each other ($P>0.05$).

BC+N and BC+A provided the lowest calcium release values (P<0.05) and similar to each other (P>0.05). There was no difference between the other groups (P>0.05).

After 14 days, HW+N, HD+N, and UB+N provided the highest calcium release values (P<0.05), whereas, BC+A provided the lowest ion release value (P<0.05). HW+A, HD+N, and BC+N showed similar values among themselves (P>0.05) and different from the other groups (P<0.05).

Table III shows the arithmetic mean and standard deviation of calcium release values (mg/L) at 24 hours and 14 days of immersion in distilled water as a function of photoactivation of pulp protection agents containing calcium hydroxide.

DISCUSSION

For this study, four light-cured calcium hydroxide-based materials were used to evaluate calcium ion release and pH values. The null hypotheses were rejected because there was variation in pH and calcium release between the groups that were subjected to the light-curing process and those that were not.

The sample size calculation in this study was based on previous studies that used the same analysis methodology [8-10]. The use of pH meter and atomic absorption spectophotometer are methods accepted in the literature, used in previous studies [8,11,12].

The aim of using calcium hydroxide is to stimulate dentin remineralization [1,10] and, for this to happen, the material must have an alkaline pH in order to demonstrate hydrogenrogen potential [8,13]. Moreover, the release of calcium into the environment is essential for remineralization to occur.

The incorporation of polymerizable methacrylates to calcium hydroxide allows the material to have lower solubility and adhesion to dentin [1-3]. However, there is a significant difference when comparing the pH value of the material with and without light-curing. This may be explained by the fact that some methacrylates do not light-cure completely [14,15]. This justifies what was found in BC+A, where the lowest pH values were found compared to the other groups evaluated.

For the pH value to be alkaline, hydroxyl ions must be present [1-3]. After the photoactivation process, the polymers formed prevent hydroxyl ion dissociation, which explains the low pH values of the groups that were light-cured [16]. Moreover, the pH value may be associated with the solubility of the material. The higher the solubility, the greater the capacity of the material to reach alkaline pH [17,18].

Table II - Arithmetic mean and standard deviation of pH values, periods of 24 hours and 14 days of immersion in distilled water, as a function of photoactivation of pulp protection agents containing calcium hydroxide

		HW+N	HW+A	HD+N	HD+A	BC+N	BC+A	UB+N	UB+A
24h	$\bar{x}$	8.78^{a}	7.31^{b}	8.84^{a}	7.30^{b}	6.33^{c}	5.57^{d}	8.95^{a}	7.35^{a}
	SD	0.27	0.16	0.31	0.21	0.33	0.12	0.38	0.39
14d	$\bar{x}$	7.35^{a}	7.21^{a}	7.39^{a}	7.29^{a}	6.23^{b}	6.35^{b}	7.40^{a}	7.50^{a}
	SD	0.18	0.40	0.15	0.38	0.43	0.18	0.20	0.75

^{a,b,c,d} Different letters on the same line indicate significant differences (P < 0.05). HW+N, non-light-cured hydroxy-cal; HW, light-cured white hydroxy-cal; HD+N, non-light cured dentin hydroxy-cal; HD+A, light-cured hydroxy-cal; BC+N, non-light-cured Biocal; BC+A, light-cured Biocal; UB+N, non-light cured Ultra-Blend Plus; UB+A, light-cured Ultra-Blend Plus; $\bar{x}$, arithmetic mean; SD, standard deviation.

Table III - Arithmetic mean and standard deviation of calcium release values (in mg/L), 24 hours and 14 days of immersion in distilled water, as a function of photoactivation of pulp protection agents containing calcium hydroxide

		HW+N	HW+A	HD+N	HD+A	BC+N	BC+A	UB+N	UB+A
24h	$\bar{x}$	40.16^{a}	3.37^{b}	39.26^{a}	4.68^{b}	0.16^{c}	0.13^{c}	41.82^{a}	4.85^{b}
	SD	10.11	0.95	13.64	1.37	0.01	0.03	14.11	1.08
14d	$\bar{x}$	4.79^{a}	0.58^{a}	4.80^{a}	0.60^{b}	0.41^{b}	0.07^{c}	4.84^{a}	0.57^{b}
	SD	0.97	0.01	1.17	0.09	0.11	0.01	1.39	0.12

^{a,b,c}Different letters on the same line indicate significant differences (P < 0.05).
HW+N, non-light-cured hydroxy-cal; HW, light-cured white hydroxy-cal; HD+N, non-light cured dentin hydroxy-cal; HD+A, light-cured hydroxy-cal; BC+N, non-light-cured Biocal; BC+A, light-cured Biocal; UB+N, non-light cured Ultra-Blend Plus; UB+A, light-cured Ultra-Blend Plus; $\bar{x}$, arithmetic mean; SD, standard deviation.

The release of calcium ions from the material into th dentinal tissue is essential for the formation of mineralized hard tissue [19]. It was observed that the materials tested after photoactivation showed lower ion values when compared to the same material without photoactivation, corroborating the study of Camilleri (2014) [20].

In an experimental in vivo study, the authors observed that the use of photopolymerized calcium hydroxide-based material did not form mineralized tissue on the dental pulp within 15 days [21,22]. This may be explained by the low release of calcium ion from these materials when subjected to light-curing.

Due to the light-curing process of these materials, there is a conversion of monomers into polymers. This conversion leads to the formation of a large marginal space between the dental tissue and the protective material, preventing it from coming into contact with moisture, leading to a lower calcium ion release.

Efficient light-curing is one of the factors for a correct conversion of monomers into polymers. Therefore, it is necessary that further studies, varying the photoactivation devices, as well as the time and power, be performed in order to question whether the light-curing process effectively interferes in the effects of these materials.

Further studies are needed to evaluate the cytotoxicity of these monomers to pulp tissue and the temperature increase caused by the light-curing process.

CONCLUSION

Photoactivation of calcium hydroxide-based dentin-pulp cements negatively interferes with the hydrogen potential and calcium ion release. However, these effects tend to stabilize after 14 days. Peculiarly, BC showed the worst values in relation to the analyses performed.

Author's Contributions

MCK: Conceptualization. PFCN, MBG, JFZ, MCK: Methodology. PFCN, JCCM, AJJ: Software. MBG, JFZ, MCK: Validation. PFCN, JCCM, AJJ, MCK: Formal Analysis. PFCN, MBG, JFZ: Investigation. JCCM, AJJ, MCK: Resources. PFCN, MCK: Data Curation. PFCN, MBG, JFZ: Writing – Original Draft Preparation. JCCM, AJJ, MCK: Writing – Review & Editing. MCK: Visualization. MCK: Supervision. PFCN, MCK: Project Administration. MCK: Funding Acquisition.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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Regulatory Statement

None.

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