

Glycerol Salicylate-based Pulp-Capping Material Containing Portland Cement

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The purpose of this study was to evaluate the water sorption, solubility, pH and ability to diffuse into dentin of a glycerol salicylate-based, pulp-capping cement in comparison to a conventional calcium hydroxide-based pulp capping material (Hydcal). An experimental cement was developed containing 60% glycerol salicylate resin, 10% methyl salicylate, 25% calcium hydroxide and 5% Portland cement. Water sorption and solubility were determined based on mass changes in the samples before and after the immersion in distilled water for 7 days. Material discs were stored in distilled water for 24 h, 7 days and 28 days, and a digital pHmeter was used to measure the pH of water. The cement's ability to diffuse into bovine dentin was assessed by Raman spectroscopy. The glycerol salicylate-based cement presented higher water sorption and lower solubility than Hydcal. The pH of water used to store the samples increased for both cements, reaching 12.59 ± 0.06 and 12.54 ± 0.05 after 7 days, for Hydcal and glycerol salicylate-based cements, respectively. Both cements were able to turn alkaline the medium at 24 h and sustain its alkalinity after 28 days. Hydcal exhibited an intense diffusion into dentin up to 40 μm deep, and the glycerol salicylate-based cement penetrated 20 μm . The experimental glycerol salicylate-based cement presents good sorption, solubility, ability to alkalize the surrounding tissues and diffusion into dentin to be used as pulp capping material.

Key Words: calcium hydroxide, dental pulp capping, pulp capping and pulpectomy agents, Raman spectrum analysis.

Introduction

Conservative treatment of deep caries with pulp exposure has been performed for centuries. In 1756, Phillip Pfaff proposed that exposed pulp could be capped by gold sheet to protect the vital tissue, in an attempt to avoid the progress of the inflammatory process and necrosis (1). The materials used for direct or indirect pulp capping should stimulate the reparative dentin formation, act against bacteria, be radiopaque, adhere to dentin and to restorative materials, be resistant to masticatory forces and seal the cavity (1).

From the use of gold sheet until today, several materials with different compositions have been used in pulp capping procedures (2-4). Calcium hydroxide-based cements, first used by Hermann in 1920 (5), have been considered the "gold standard" cements for pulp protection. Calcium hydroxide (CH) powder can be mixed with water or incorporated into a resinous matrix, generally a salicylate ester, to obtain a self-setting cement. CH-based cements release calcium and hydroxyl ions, encouraging the formation of a reparative dentin (6) and killing bacteria (7). For indirect pulp capping it is important that cements be able to diffuse into dentin in order to signal the pulp cells.

More recently (8), mineral trioxide aggregate (MTA) was introduced in Endodontics and showed better

clinical performance used as direct pulp capping agent than CH-based cements (9). MTA presents a chemical composition similar to Portland cement clinker, with the addition of bismuth oxide to improve its radiopacity (8). MTA's mechanism of action is similar to that of calcium hydroxide, as its interaction with water produces calcium hydroxide during the cement setting. In comparison to CH-based cements, MTA has lower solubility and higher mechanical properties and calcium ion release (3). However, pure MTA as pulp capping is difficult due to the longer setting time and poor handling. To take advantage of the beneficial properties of MTA and to produce a cement with improved handling properties, MTA and Portland have been incorporated into non-aqueous vehicles, such as acrylic monomers or resins (2,10,11). To date, no pulp-capping materials based on salicylate resin including MTA or Portland in their composition have been developed.

Salicylate compounds are widely used in the pharmaceutical industry and have anti-inflammatory potential (12). Aiming to produce a low-solubility salicylate, a glycerol salicylate was synthesized from the transesterification reaction of glycerol and methyl salicylate (13). This glycerol salicylate can present up to three reactive groups to chelate calcium ions and set, instead of the two reactive sites present in dissalicylates usually used

in commercial CH-based pulp capping cements. Glycerol salicylate molecules are composed of three salicylates bonded to a central glycerol, which confers high viscosity to the resin. Thus, a diluent such as the methyl salicylate, is required to adjust resin viscosity and make it suitable for clinical use.

This study evaluated the water sorption, solubility, pH and ability to diffuse into dentin of experimental glycerol salicylate-based pulp-capping cement in comparison to conventional calcium hydroxide-based cement.

Material and Methods

An experimental cement was developed consisting of 60% glycerol salicylate resin, obtained according to Portella et al. (13), 10% methyl salicylate (Vetec Química Fina Ltda, Duque de Caxias, RJ, Brazil), 25% calcium hydroxide (CH; Biodinâmica, Iporã, PR, Brazil) and 5% Portland cement (CP IV-32; Votorantim Cimentos, Esteio, RS, Brazil). The above concentrations refer to the weight percentage. Liquid (glycerol salicylate resin and methyl salicylate) and powder (calcium hydroxide and Portland cement) reagents were weighed on an analytical scale, according to the above concentrations and hand mixed on a glass plate. Fresh mixtures of the cements were inserted into silicon matrix to produce sample discs of 10 ± 0.1 mm diameter and 1.0 ± 0.01 mm thick. Samples remained stored at 36 °C in a humid cabinet up to the complete setting of the materials. The experimental cement's sorption, solubility, pH and cement diffusion into dentin were compared with those of a commercial pulp capping cement (Hydcal; Technew Com. Ind. Ltda, Rio de Janeiro, RJ, Brazil). Hydcal was manipulated according manufacturer instructions, by hand mixing equal amounts of base and catalyst pastes.

Sorption and Solubility

Sorption and solubility were evaluated as described in a previous study (14). Three samples were soaked in 10 mL of distilled water at 36 °C for 7 days. The volume (V) of the specimens was calculated before immersion. The mass of the specimens was determined before (m_1), immediately after water immersion (m_2) and after drying (m_3). Before m_1 and m_3 were assessed, the specimens were kept in desiccators and weighed every 24 h, until a constant mass was obtained. Water sorption (W_{sp}) and water solubility (W_{si}) were calculated according to the following equations, and the mean values of three specimens were recorded:

$$W_{sp} = (m_2 - m_3) / V$$

$$W_{si} = (m_1 - m_3) / V$$

pH Analysis

The pH of storage water from the sorption and solubility tests, and water from other samples stored in the same

way for 24 h and 28 days, were evaluated using a digital pHmeter (DM-23; Digicrom Analytical Ltda, São Paulo, SP, Brazil). Three measurements were performed per group for each period, at 24 h, 7 days and 28 days. The initial pH of the distilled water was 7.79. Formation of crystals during water storage in the pH test was observed by direct visualization. The crystals were characterized by Raman spectroscopy using a 785 nm laser (Senterra; Bruker Optics, Ettlingen, Germany).

Raman Analysis of Cement Diffusion

The buccal enamel of two bovine incisors was ground to expose the superficial dentin. The dentin was prepared with 600-grit SiC paper for 30 s in running water. The experimental cements were manipulated and applied over the exposed dentin, and then restored using a self-etching adhesive system (Clearfil SE Bond; Kuraray America Inc., New York, NY, USA) and a micro-hybrid composite resin (Charisma; Heraeus Kulzer GmbH, Hanau, Germany) to simulate clinical situation. The teeth were stored in simulated body fluid (SBF) (15) at 36 °C for 28 days. SBF had the following ion concentration, in mol/m³: Na⁺ (142.0), K⁺ (5.0), Mg²⁺ (1.5), Ca²⁺ (2.5), Cl⁻ (147.8), HCO₃⁻ (4.2), HPO₄²⁻ (1.0) and SO₄²⁻ (0.5). The pH was adjusted to 7.4.

The teeth were sectioned longitudinally to the crown axis at the center of the restoration. The hemisections were sonicated for 180 s to remove debris and the specimens were analyzed by micro-Raman spectroscopy (Senterra, Bruker). Mapping areas of 100 x 100 μm of dentin underneath the cement (Fig. 1) were analyzed. The samples were irradiated on 100 points. Each point was irradiated 2 times for 5 s by a 785 nm 100 mW laser. The spectra were obtained, the integrals of the 1,084 and 1,034 cm⁻¹ peaks were determined for each point and the values were plotted onto a graph. The 1,084 and 1,034 cm⁻¹ peaks relate to calcium hydroxide chemical bonds (13) and to bands of salicylate resin chemical bonds (16), respectively.

Statistical Analysis

Differences in sorption and solubility were analyzed by *t* tests, and the differences in pH values were analyzed using two-way ANOVA and Tukey's test. The significance level was set at 5%.

Results

Table 1 shows the water sorption and solubility and pH of water storage after different periods. There were significant differences in sorption ($p=0.015$) and solubility ($p=0.049$) between the materials, glycerol salicylate-based cement exhibiting higher water sorption and lower solubility values compared with Hydcal. Both cements were able to alkalize the medium at 24 h and sustain the alkalinity after 28 days.

The presence of crystals was observed in the pH test water for the glycerol salicylate-based cement after 28 days. One of these crystals and its Raman spectrum are shown in Figure 2. The micro Raman analysis of the crystal exhibits 550, 665, 836, 886, 1050 and 1159 cm^{-1} bands (Fig. 2c).

Figure 3 shows representative spectra of non-infiltrated dentin and dentin infiltrated by cement. Raman band shift presented 804, 962, 1034 and 1084 cm^{-1} peaks. Figure 4 shows the intensity of the 1084 and 1034 cm^{-1} peaks on the mapped dentin adjacent to the cement after sample storage in SBF. The sample of Hydcal presented an intense diffusion of cement up to 40 μm depth. The tooth that received the glycerol salicylate-based cement showed cement penetration up to 20 μm but this diffusion was not consistent for the entire region, as in the Hydcal sample.

Discussion

In this study, novel pulp-capping cement based on a salicylate resin, calcium hydroxide and Portland cement was developed and had the physicochemical properties

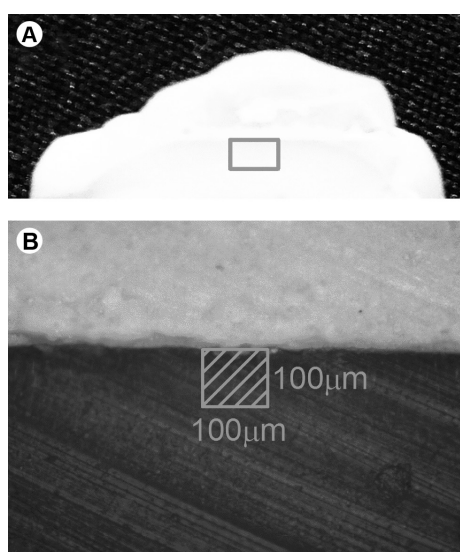


Figure 1. A: Transversal section of tooth sample showing the tooth pulp/capping material interface. Area analyzed by micro-Raman spectroscopy (square). B: Higher magnification (50x) of the analyzed area, showing the mapped region.

Table 1. Means and standard deviations of sorption, solubility and pH observed for the tested pulp-capping materials

Cement	Sorption ($\mu\text{m}/\text{mm}^3$)	Solubility ($\mu\text{m}/\text{mm}^3$)	pH		
			24h	7 days	28 days
Hydcal	342.97 \pm 85.95B	500.36 \pm 133.59A	11.56 \pm 0.07Ac	12.59 \pm 0.06Aa	12.04 \pm 0.05Ab
GSR*+CH+Portland	547.95 \pm 9.14A	283.16 \pm 10.93B	10.89 \pm 0.01Bc	12.54 \pm 0.05Aa	11.19 \pm 0.07Bb

*GSR: Glycerol salicylate resin. The means followed by different capital letters indicate significant differences ($p < 0.05$) in the same column. The means followed by lowercase letters indicate significant differences in pH evaluation ($p < 0.05$) in the same line. The initial pH of the used distilled water is 7.79.

of sorption, solubility, pH and the ability to diffuse into dentin compared with Hydcal (a commercial calcium hydroxide-based pulp-capping cement of similar chemical composition). Hydcal is basically composed of salicylate resin and calcium hydroxide. Prior the development of glycerol salicylate-based pulp-capping cement, a set of Portland cement concentrations was tested, ranging from 5 to 15%. Calcium hydroxide was replaced by Portland cement in the mixture. Due to the low availability of Ca^{2+} in groups with higher Portland contents, the setting times increased and cement became too dark (since grey Portland cement was used to formulate experimental cements in pilot studies). Furthermore, the cement without calcium hydroxide did not set. Based on that, it was assumed that 5% (in weight) of Portland cement represented the concentration that exerted less influence on the experimental cement's properties (setting time and color).

Glycerol salicylate-based cement is set by an acid-base reaction between calcium hydroxide and salicylate resin. Calcium is chelated by salicylate, forming a calcium salicylate salt (13). This is a similar hardening process to that of available commercial calcium hydroxide cements. Nevertheless, the majority of current cements are composed of a dysfunctional salicylate ester (21,22), unlike the resin used in this study, which presents up to three salicylates in its molecule, which theoretically allows the binding of up to three calcium ions by the same molecule, contributing to the lower solubility of glycerol salicylate-based cement in comparison to Hydcal. Additionally, in contact with humidity Portland cement hydrates, forming a hydrated calcium silicate matrix and calcium hydroxide. Portland hydration and crystal formation occurred when the glycerol salicylate-based cement was immersed in water. Silicate bending bands at 665 cm^{-1} and symmetrical stretching bands at 800-1050 cm^{-1} , characteristic of calcium silicate hydrate are presented in Figure 2. Band 1050 cm^{-1} could be also present in glycerol resin. However, the crystal was not analyzed in contact with glycerol resin, indicating the correspondence of 1050 cm^{-1} to calcium silicate hydrate. The 550 cm^{-1} and 1159 cm^{-1} bands can be assigned to alite and belite, and to bassanite, respectively, probably present due an

incomplete hydration of Portland cement (17,18). Water uptake promoted by the Portland cement could lead to hydraulic setting, promoting the formation of a calcium silicate hydrate matrix (23), which has the potential to stimulate mineral deposition in

contact with biological fluids, such as dentinal fluid. In this way, the chemical features of cement after hydration lead to a bioactive surface that possibly may help to form reparative dentin, stimulating the mineralization of tissues.

Aside from higher water sorption of the glycerol salicylate-based cement, its solubility was lower than Hydcal. The great amount of salicylate resin used in the experimental cement could explain these patterns of sorption and solubility. The salicylate resin matrix, present on both cements, contributes to elevated water sorption and solubility, insofar as salicylate-based cements are prone to absorbing water (24) and solubilizing (11,25). Higher water sorption could lead to cement swelling, offsetting the negative dimensional changes that occur with the cement's solubilization. However, the dimensional changes of the cements after water sorption were not assessed. As in a previous study (14), neither the ISO 6876 (Dental root canal sealing materials) nor ISO 9917 (Water-based cements) parameters were adopted to measure sorption and solubility, since both tested cements are resin-based and the aforementioned parameters are designed for water-based cements and root canal sealing materials. The lack of an international standard method to assess the properties of resin-modified calcium silicate cements has already been pointed out (2).

Fluid stability is important for maintaining an effective seal against bacteria and preventing the development of defects under restorations, which could impair the restoration's resistance (26). In a previous study, the authors stated that traditional calcium hydroxide-based cements dissolve over time in a short period (i.e., 2 years) (2). The

low solubility levels of glycerol salicylate-based cement followed by cement diffusion in dentin and calcium-silicate crystal formation could mitigate this shortcoming. Moreover, Portland cement has recognized bioactivity, stimulating the production of an apatite structure over its surface in contact with tissues (27) and allowing the expression of mRNAs from a dentin-specific protein and a non-collagen protein involved in mineralization (28). These properties could accelerate the development of a mineralized barrier over the exposed pulp.

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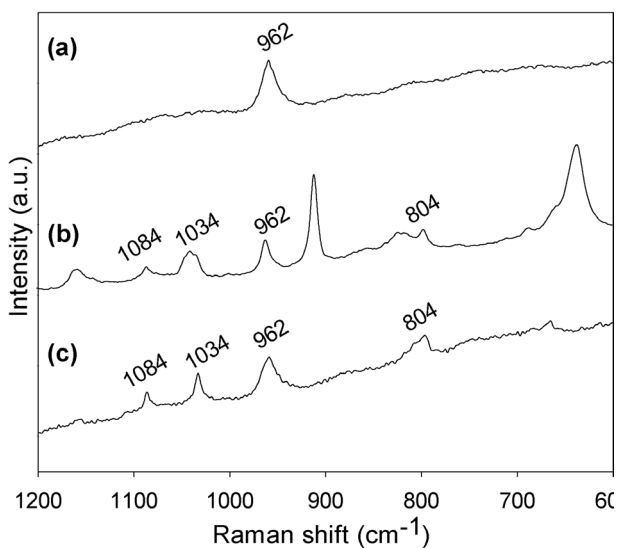


Figure 3. Representative spectra of dentin that did not receive a pulp-capping material (A), that received Hydcal (B) and that received the glycerol salicylate-based cement (C).

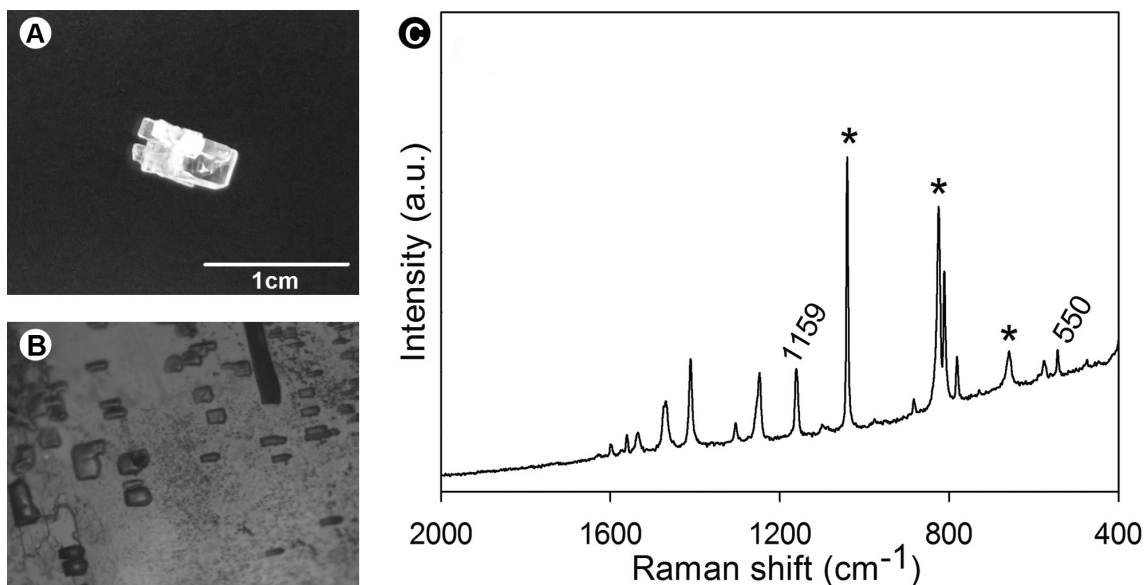


Figure 2. A: Crystal formed after 28 days of water storage. The maximum length of the crystals was approximately 6 mm. B: 50x magnification of crystals obtained using an optical microscope coupled to Raman equipment. C: Raman spectrum obtained from crystals formed during the sorption and solubility tests.

Recently, a practice-based randomized clinical trial evaluated the success of direct pulp capping in permanent teeth performed with both calcium hydroxide and MTA as pulp-capping cements, and estimates that the failure rates after 24 months reached 31.5% and 19.7% for teeth pulp capped with calcium hydroxide and MTA, respectively (9). These results corroborate those of Mente et al. (29), where MTA showed a higher success rate than calcium hydroxide (78% vs. 60%). For direct pulp capping, both materials present the same success rates and ability to stimulate reparative dentin formation (30). Thus, to improve the handling properties of MTA or Portland and optimize its results on direct pulp capping, MTA was incorporated on a salicylate resin matrix (10,11).

The anti-inflammatory potential of salicylates is probably present in salicylate resins. Salicylates are capable of inhibiting the action of cyclooxygenase-2 and consequently the synthesis of prostaglandin (12). This anti-inflammatory property is desirable for cements that will be placed in contact with pulp tissue to reduce the inflammatory process provoked by caries progression, in an attempt to prevent the development of pulp necrosis and/or postoperative sensitivity.

Glycerol salicylate-based cement and Hydcal were able to elevate water pH above 8.5 after 24 h of immersion. This

is the optimum pH for alkaline phosphatase, which plays an important function in tissue mineralization (31). The alkalinity of both cements increased to pH=12 at 7 days and decreased slightly after 28 days, but both cements sustained the high pH. The increase in pH is probably due to hydroxyl ions being released from the calcium hydroxide originally present in the cements' compositions and from the calcium hydroxide formed from the hydration of Portland cement.

Experimental glycerol salicylate-based Portland cement did not show the same diffusion pattern into dentin as Hydcal does, when evaluated using Raman to map the intensity of 1,084 and 1,034 cm^{-1} peaks related to the chemical bonds of calcium hydroxide and salicylate resin, respectively. For the non-infiltrated dentin, the peak at the 962 cm^{-1} band corresponds to symmetric stretching mode of phosphate (19). In the spectrum of the dentin adjacent to the cement, other peaks were noticed, like 1,084 cm^{-1} , which is present for calcium hydroxide (13), and at 1,034 and 804 cm^{-1} , which refer to bending in plane CH mode (16,20) and to the stretching $\text{C}_{\text{aromatic}}\text{-COOH}$ of salicylate resin (16). The diffusion of cement components, such as calcium hydroxide, into dentin is expected to be beneficial for reparative dentin formation. Hydcal presents ethyl toluene sulfonamide in its composition, a plasticizer that could decrease cement viscosity and improve penetration into dentin. The glycerol

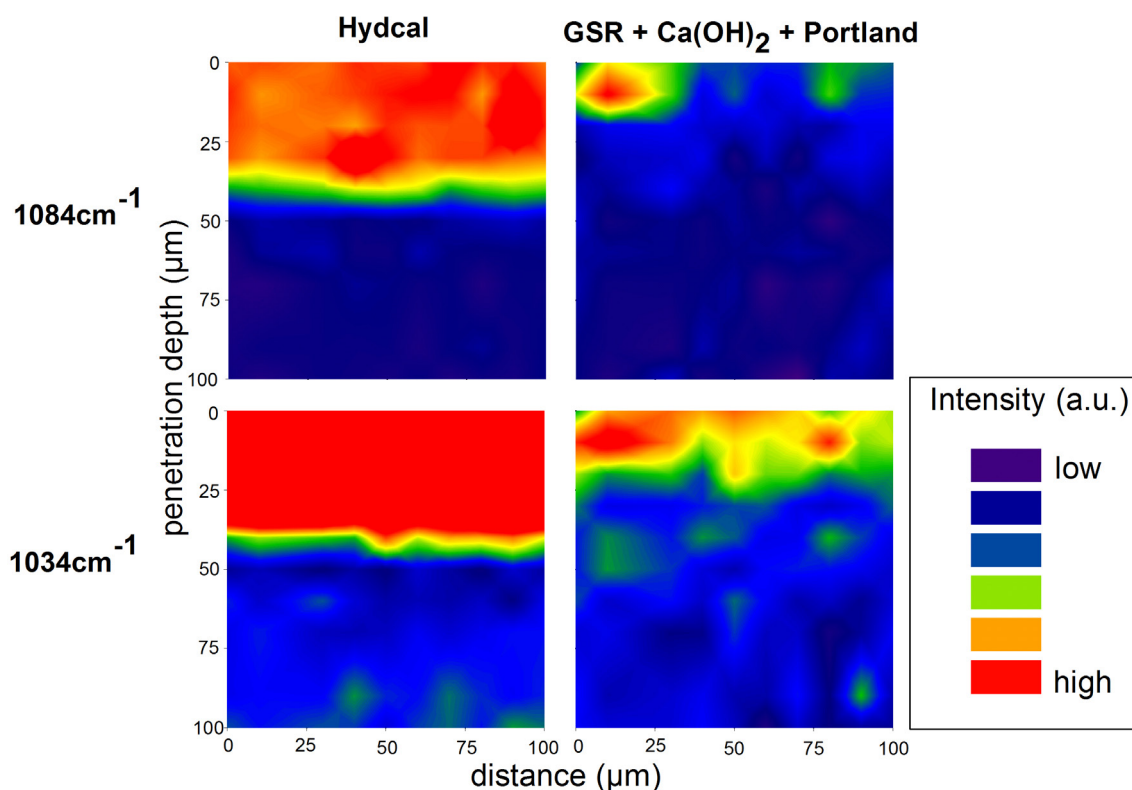


Figure 4. Raman intensity of the 1,084 (referring to calcium hydroxide diffusion) and 1,034 cm^{-1} (referring to salicylate resin) peak areas measured on each point of the mapped area, as a function of cement's diffusion.

salicylate-based cement is a model cement, and its other physical-chemical properties should be adjusted before *in vivo* tests. Future research should be conducted focusing on adjusting the setting time and radiopacity.

The experimental glycerol salicylate-based cement presents good sorption, solubility and ability to alkalinize the surrounding tissues and diffuse into dentin to be used as pulp capping material.

Resumo

O objetivo deste estudo foi avaliar a sorção e solubilidade em água, pH e habilidade de difusão na dentina de um cimento para capeamento pulpar à base de glicerol salicilato e compará-lo a um cimento comercial para capeamento pulpar à base de hidróxido de cálcio (Hydcal). Um cimento experimental contendo 60% de resina de glicerol salicilato, 10% de salicilato de metila, 25% de hidróxido de cálcio e 5% de cimento Portland foi formulado. Sorção e solubilidade em água foram determinadas a partir da alteração na massa de espécimes antes e após a imersão em água destilada por 7 dias. Discos dos cimentos foram armazenados em água destilada por 24h, 7 dias e 28 dias e o pH da água foi aferido após cada período. A habilidade de difundir-se no interior de dentina bovina foi avaliada por espectroscopia Raman. O cimento à base de glicerol salicilato apresentou maior sorção e menor solubilidade em comparação com o Hydcal. O pH da água de armazenamento dos espécimes aumentou para ambos os cimentos, chegando a $12,59 \pm 0,06$ e $12,54 \pm 0,05$ após 7 dias, para o Hydcal e o cimento à base de glicerol salicilato, respectivamente. Os cimentos foram capazes de promover a alcalinização do meio após 24h e sustentaram a alcalinidade após 28 dias. Hydcal exibiu intensa difusão na dentina até 40 μm de profundidade e o cimento à base de glicerol salicilato penetrou 20 μm . O cimento experimental à base de glicerol salicilato apresentou adequada sorção, solubilidade, habilidade de alcalinizar o meio e difundir-se no interior da dentina para uso como um material para capeamento pulpar.

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