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Preclinical effectiveness of a novel pulp capping material

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Introduction

Calcium hydroxide is the most commonly-used pulp capping material because it can chemically cauterize the bleeding pulp and stimulate pulp healing and mineralization (1). However, calcium hydroxide also has significant limitations: It lacks the sealing properties necessary to prevent bacterial microleakage (2). It is highly-soluble and can easily dissolve over time (3). To help address these limitations, visible light-curing resin was added to calcium hydroxide to create visible light-curing resin-modified calcium hydroxide materials (VLCCHs). Compared to calcium hydroxide, the VLCCHs have improved mechanical properties and handling characteristics (4). However, VLCCH also has deficiencies, particularly its lack of adhesive and dentin remineralization properties. To address the deficiencies, a resin-based calcium phosphate cement (RCPC) with superior adhesive properties has been developed (5-8). RCPC can remineralize mineral deficient dentin, unlike other materials used for direct pulp capping (7,9,10).

The direct pulp capping of exposed dental pulps with dental materials have shown that reparative dentin healing response, sometimes known as dentin bridging, forms in animal (11,12) and human (13) teeth. The overall success rate of pulp-capping is approximately 33% (14). Thus, improved materials are needed for more successful procedures. However, it is unclear if RCPC is superior to VLCCH to stimulate reparative dentinogenesis and to prevent bacterial microleakage.

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The present study compared the effectiveness of an experimental RCPC and VLCCH to induce reparative dentinogenesis and prevent bacterial microleakage following pulp capping in dog and ferret teeth.

Materials and Methods

Animal welfare

The use of three mongrel dogs and six ferrets was approved by the Animal Care and Use Committees at the Universities of West Virginia and Michigan.

Pulp exposure of non-inflamed dog teeth

Three mongrel dogs, approximately 18 to 36 months of age were anesthetized. Rubber dam was not placed. The tooth surfaces were cleaned and polished, and then disinfected with chlorhexidine. Under cotton roll isolation with high volume suction, midcrown Class V buccal or labial cavities were prepared in the maxillary and mandibular premolars and the first molar using new #330 carbide burs under an air-sterile water spray. The preparations were bordered by enamel and were large enough to extend inside the mesial and distal surfaces of the tooth and into the inner one third of dentin. The enamel margins were beveled prior to exposure of the pulp. In the center of the cavity a pulp exposure, approximately 0.5 mm to 1 mm in diameter, was made under a spray of sterile saline solution. Care was taken to prevent the bur from penetrating into the pulpal tissue. A flat depression was prepared in dentin surrounding the exposure site to allow adaptation of materials. The exposure site was thoroughly irrigated with sterile saline and hemorrhage was controlled with sterile cotton pellets.

Pulp exposure of inflamed and non-inflamed ferret teeth

Six adult male ferrets were anesthetized. Reversible pulpitis was induced in 16 ferret teeth by removing the cusp tip to create small ($\approx 200 \mu\text{m}$) exposures. $2 \mu\text{g}$ of *Salmonella typhimurium* lipopolysaccharide (LPS) in $2 \mu\text{L}$ saline was injected into the pulp to a depth of about 1mm. The teeth were obturated with light-cured composite resin material (Tetric Ceram, Ivoclar Vivadent, Schaan, Liechtenstein). Three days later, the animals were anesthetized. An additional eight teeth did not have pulpitis inducing procedures. A 0.5 mm sterile bur was used to expose the pulp and partial coronal pulpectomy was performed. The exposures were rinsed with sterile saline and dried with paper points to achieve hemostasis. A flat depression was prepared in dentin surrounding the exposure site to allow adaptation of RCPC. The exposure site was thoroughly irrigated with sterile saline and hemorrhage was controlled with sterile cotton pellets.

Capping of pulp exposures

Twelve teeth in each dog (Table 1a) and four teeth in each ferret (Table 1b) were randomly assigned to receive either RCPC (Paffenbarger Research Center, Gaithersburg, MD) or VLCCH (VLC Dycal, Dentsply Int., Milford, DE, USA). The RCPC was freshly prepared by hand-mixing Paste #1 and Paste #2 in a 50%/50% ratio (7). Paste #1 of the two-paste cement consisted of pyromellitic glycerol dimethacrylate as the acidic, adhesive resin, water, anhydrous dicalcium phosphate, sodium hexafluorosilicate, camphorquinone, and benzoyl peroxide. Paste #2 contained ethoxylated bisphenol-A dimethacrylate, calcium-enriched tetracalcium phosphate and N,N-dimethylaminophenethanol. The mixed RCPC paste was placed over the site of the pulp exposure using gentle pressure to adapt it to the surrounding hard tissue. The VLCCH was prepared according to manufacturer's instructions. Both RCPC and VLCCH were light-cured for 20 seconds. Phosphoric acid gel (37%) was used to etch the surrounding enamel and dentin, while avoiding acid contact with the dental material as much as possible. This was followed by a 10 second rinse with sterile water. For dog teeth, two drops

of a PMGDM-HEMA-based acetone containing bonding agent (Paffenbarger Research Center, Gaithersburg, MD) were dispensed in a dappen dish, two coats were applied with a small brush to the capping material and cavity walls and the bonding agent was light-cured for 10 seconds. Composite resin (TPH, Dentsply Int., Milford, DE, USA) was inserted in the cavity and light-cured (Elipar Highlight 3M ESPE, Seefeld, Germany). The final restoration was finished and polished flush with the beveled enamel margins using abrasive discs. The ferret teeth were restored after applying the bonding agent Excite with the light-cured composite resin Tetric Ceram (both from Ivoclar-Vivadent, Schaan, Liechtenstein).

Euthanization of animals

One dog was euthanized at 7 days, one at 28 days, and one at 90 days. The ferrets were sacrificed after 45 days (15).

Processing and histologic analysis of teeth

The teeth were processed for histology and embedded in paraffin wax, sectioned into four micrometer thick sections and stained with hematoxylin/eosin. The histology of the reparative dentin and cellular responses were analyzed with a light microscope. The cellular response were rated on a scale from 0 (no adverse reaction) to 4 (micro-abscess) following ISO guide lines (16). The histology of reparative dentin was analyzed similar to a previous study by Rutherford and Gu (15). The bacterial microleakage of the 90 day dog teeth were evaluated following Brown & Brenn staining (17).

Statistical analyses

The bacterial and inflammation scores of the dog teeth were analyzed by the nonparametric Mann-Whitney rank sum test, $p < 0.05$. For both dog and ferret teeth, the formation of a dentin bridge was analyzed by Fisher's exact test, $p < 0.05$.

Results

Pulp capping of dog teeth

After 7 days no reparative dentin was found with either pulp capping material. Inflammation scores were low (Fig. 1a) except for three teeth.

After 28 days, reparative dentin was found in 6/8 teeth RCPC-capped. Two teeth were not rated; one lacked a confirmed exposure, the other had an artifact. No reparative dentin was detected in the four VLCCH-capped pulps (Fig. 1a). They showed mostly chronic inflammation.

After 90 days, seven of the rated RCPC-capped pulps had reparative dentin formation (Figure 2), while the VLCCH-capped pulps had none (Table 1a). Tooth number eight was excluded as pulpal exposure was not observed. The 90 day inflammation scores (Fig. 1a) were significantly different between VLCCH- and RCPC-treatments (Mann-Whitney rank sum test, $p = 0.006$). 57% of the RCPC-treated teeth, as opposed to 25% of the teeth treated with VLCCH, were free of bacteria (Fig. 1b); however, the difference between the groups considering all bacterial responses was not statistically significant.

Pulp capping of ferret teeth

All of the ferret non-inflamed teeth treated with RCPC or VLCCH had reparative dentin (data not shown). In 75% of the inflamed teeth, RCPC was capable of stimulating reparative dentin (Table 1b). Similar to the dog teeth (Fig. 2), the ferret reparative dentin had an irregular pre-dentin-like appearance aligned with a layer of odontoblast-like cells. In most experimentally

inflamed teeth, but not in the non-inflamed teeth, residual inflammation was evident at 45 days (Fig. 1b). Histomorphometric analyses revealed that at 45 days the incomplete coverage of the exposure with dentin was found in all inflamed teeth, while reparative dentin more completely bridged the site of pulp exposure in non-inflamed teeth treated with RCPC or VLCCH (Table 1b). Significantly more reparative dentin formed in response to RCPC than VLCCH in the inflamed teeth (Table 1b).

Discussion

New pulp capping materials are needed to improve the clinical success of pulp capping treatments which are generally not very successful (14). This study is the first to compare VLCCH and RCPC for the direct pulp capping of inflamed and non-inflamed dental pulps.

The pulp capping of dog teeth with VLCCH exhibited high inflammation scores, and a high degree of bacterial invasion. None of these teeth had reparative dentin. These results contrast previous studies of VLCCH or another similar light-cured calcium hydroxide based material (4,18-20). The reasons for the high inflammation were lack of material biocompatibility and more importantly bacterial microleakage (21). One reason for the lack of reparative dentin was the high amount of bleeding, which is known to reduce the success of pulp capping procedures (22). The disastrous results of the VLCCH appear similar to that of bonding agents that have been used for pulp-capping (18,23).

RCPC was also more successful at stimulating reparative dentin and generally better preventing microleakage in comparison to VLCCH in dog teeth. During pulp capping, it was noted that RCPC stopped the bleeding more quickly, probably due to an initial high pH (6).

The ferret study was added to assess whether RCPC would also be effective on inflamed teeth. Previously, only a few studies have been conducted capping inflamed dental pulps (15,24, 25). Generally, successful pulp-capping with calcium hydroxide is achieved in 90% of the cases, provided that the treatment is restricted to non-inflamed pulps and the material is sealed to prevent microleakage (26). Previous data had demonstrated that the coronal pulpitis induced in this model is reversible (15) and that the pulp is capable of healing through reparative dentinogenesis (25). In the present study, more reparative dentin formed in RCPC-treated teeth than in teeth capped with VLCCH.

In conclusion, RCPC has been shown to induce reparative dentinogenesis in two animal models of pulp exposure. The results suggest that RCPC might be used as a therapeutic material for the capping of vital and inflamed pulps in teeth exposed through trauma or caries.

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Figure 1.

Figure 1a. Inflammation scores of dog teeth. At 90 days the inflammation scores between RCPC and VLCCH were significantly different, $p = 0.006$, Mann-Whitney Rank Sum Test.

Figure 1b. Bacterial scores of ferret teeth. At 45 days the bacterial scores between RCPC and VLCCH were significantly different, $p = 0.006$, Mann-Whitney Rank Sum Test.

Figure 2.

Image of a dog tooth after 90 days showing complete dentin bridging. Some partly destroyed pulp tissue and 2 dentin chips are also shown.

Table 1

Table 1a: Reparative dentin (RD) formation in RCPC- and calcium hydroxide (VLCCH)-treated dog teeth after 7 d, 28 d, or 90 d.			
Pulp capping material	Teeth with reparative dentin		
	7 d	28 d	90 d
VLCCH	0	0	0
RCPC	0	6/8	7/7*
Table 1b: Response of inflamed ferret teeth to RCPC and calcium hydroxide (VLCCH) treatment.			
	Inflamed teeth with reparative dentin	Percent of exposure filled*	Area of reparative dentin
VLCCH	4/8	21 % ± 6 %	0.03 ± 0.008 mm ²
RCPC	6/8	78 % ± 11 %	0.18 ± 0.06 mm ²

* One tooth was not exposed. After 90 d, RCPC induced RD in significantly more teeth than VLCCH (Fisher's exact test $p = 0.003$).

* Significantly more reparative dentin formed in RCPC treated teeth than calcium hydroxide treated teeth (Fisher's exact test, $p < 0.05$). Values are mean ± standard deviation as a measure of the standard uncertainty. 10 microscopic fields in each of 8 sections were analyzed from each tooth.