

A HISTOLOGIC ASSESSMENT OF A HYBENX® ORAL TISSUE DECONTAMINANT IN VITAL PULP THERAPY IN DOGS

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The aim of this study was to assess HYBENX® Oral Tissue Decontaminant (HOTD) in treating vital pulp exposure in a canine model. The use of HOTD solution was compared to an accepted and standard regimen for vital pulp exposure, an application of a commercial calcium hydroxide product (Ca(OH)₂). Both control and experimental treatments were followed by restoration with a commercial zinc oxide and eugenol obtundant intermediate restorative material and thermal insulator (ZOE). At 7 days there was 100% pulp vitality with HOTD and 50% with Ca(OH)₂. New dentin formation was seen in 62.5% of the HOTD treated pulps and none of the Ca(OH)₂ treatment group. The vital pulp exposures at day 21 post treatment with HOTD also showed significant improvement over Ca(OH)₂ in the presence of odontoblasts, new dentin formation and pulp survivability. The presence of odontoblasts and new dentin was noted in 71% of the HOTD cases versus 50% of the survivable Ca(OH)₂ cases. Furthermore, 100% of HOTD cases had vital pulps versus 62.5% of Ca(OH)₂ cases. The 60-day specimens of both experimental and control techniques exhibited histologically similar appearances and were similar in outcomes. HOTD treatment at day 7 showed a significant positive difference, both in the formation of new dentin and tooth vitality. HOTD proved better for the post 21-day specimens and equivalent for the 60-day pulp specimens with no evidence of untoward tissue reactions or results.

Periodontal disease and dental caries are the two most common oral diseases. The cause of both of these diseases is bacterial, the bacteria being found in a biofilm, the dental plaque. The bacteria in the dental plaque are capable of reacting with refined sugars in the diet and forming acid that will cause dental caries (1, 2). The present study was conducted to assess the ability of a HYBENX® Oral Tissue Decontaminant (HOTD) (EPIEN Medical, Inc., St. Paul, MN 55110 USA) to complement the treatment of vital dental pulp exposures, a common consequence of trauma, dental caries or the attempt at debridement of dental caries. This is recognized as a therapeutic problem in dogs (3). Because HOTD

performs selective, self-limited denaturation and coagulation of superficial biological structures on tissue and environmental surfaces using proprietary liquid desiccating agents (4) it was postulated that it could help maintain the vitality of the dental pulp to preserve a vital tooth that could be restored and maintained in the dental arch. Exposures of vital pulp tissue must be immediately treated with a substance that will debride the pulp tissue of contaminating biofilm and thus prevent acute inflammation leading to irreversible pulpitis, as well as stimulate odontoblasts to form reparative dentin and repair the area of exposure. Our hypothesis was that the HOTD could accomplish this.

Key words: HYBENX® Oral Tissue Decontaminant, denaturation, coagulation, pulp capping, canine teeth, Dycal®, IRM®, biofilm

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MATERIALS AND METHODS

Ethical approval for the animals used in the research was obtained through the Institutional Animal Care and Use Committee (IACUC) at LyChron, LLC, Mountain View, CA and the VA Medical Center, Minneapolis, MN.

Direct Pulp Capping

This study is an assessment of the use of HOTD in the treatment of direct pulp capping in a canine model of vital pulp exposure. Left and right maxillary premolars and first molar, and left and right mandibular premolars, first and second molars were chosen as study teeth in three dogs. Problems with the condition of teeth resulted in using 9-10 teeth per animal. According to protocols established in the literature (5-8), the dogs were anesthetized with a general anesthetic regimen and intubation with an endotracheal tube, and a throat pack of folded gauze placed in the posterior oral pharyngeal space. All personnel used appropriate protective equipment.

An exposure of the vital pulp was accomplished for the selected teeth by a penetration just through the facial surface of the tooth occlusal to the cemento-enamel with a high-speed dental hand-piece and a number 35 inverted cone dental bur. The bur causing the exposure was extended into and through the pulp through the buccal surface until the lingual surface of the pulp chamber was encountered so that at microscopic examination the pathologist could verify that the lesions created at the control and the test site were of equal severity. One bur was used for all teeth in all animals so the bur at the time of use was contaminated and no effort was made to clean any teeth prior to pulpal exposure. The purpose was to have an acute traumatic and contaminated wound of the pulp. All teeth bled at time of bur penetration.

For the control treatment, the pulpal exposure was rinsed with saline from an irrigation syringe after penetration, lightly air-dried and then capped by a placement of commercial calcium hydroxide product Ca(OH)₂ (DENTSPLY DYCAL®, DENTSPLY International, Inc., York, PA 17405 USA), a long standing standard procedure (9). A commercial zinc oxide and eugenol obtundant intermediate restorative material and thermal insulator (ZOE) (DENTSPLY IRM®, DENTSPLY International, Inc., York, PA 17405 USA) was then placed in the pulpal access hole per manufacturer's directions. The

experimental site was prepared exactly like the control site with the exception that after penetration no Ca(OH)₂ was used. The exposure was irrigated with HOTD via irrigation syringe directly onto the pulpal tissue, then rinsed with saline and sealed with the ZOE as with the control. The only difference between the control and experimental sites was the use of calcium hydroxide in the control and the use HOTD solution in the experimental sites. One dog was euthanized with the tissues subjected to perfusion fixation immediately post-mortem at 7 days, one at 21 days, and one at 60 days post operatively. The maxillae and mandibles were harvested with dentition and gingiva

intact and placed into formalin. When received at the Hard Tissue Research Laboratory of the University of Minnesota School of Dentistry, the experimental teeth were removed from the surrounding tissue with a water-irrigated diamond coated blade (EXAKT® Sage-Schliffe, EXAKT Apparatabau, Niederstadt, Germany) without damaging the teeth. The teeth were sectioned with this same instrument through the experimental pulp penetration.

Histological Processing

Specimens were dehydrated with a graded series of alcohols, infiltrated with a light curing embedding resin (Technovit 7200 VLC, Heraeus Kulzer, Hanau, Germany) and polymerized in a light curing system in which the tissues never exceeded 40C (EXAKT Apparatabau, Niederstadt, Germany). Without decalcification, sagittal sections of the teeth with pulp exposures were selected through the buccal-lingual aspect through the point of pulp penetration. Specimens were cut to a thickness of 150µm and polished to a final thickness of 45-65µm in an EXAKT polishing and micro-grinding system followed by 3 micron alumina polishing paste (10). Specimens were stained with Stevenel's blue and Van Gieson's picro fuchsin.

Histological Analysis

Two investigators (MDR and HSP) analyzed the histological specimens. The investigators were blinded as to the treatment of the pulp-capping specimens. They analyzed several parameters in the direct pulp capping study: fixation of the pulp, pulp penetration by the contaminated bur, treatment material contact with pulp tissue, vitality of the pulp, presence of odontoblasts and new dentin formation.

RESULTS

Seven-day, 21-day and 60-day specimens were evaluated.

Seven-Day Specimens

Sixteen specimens were included in the 21-day study; eight each had been treated with Ca(OH)₂ and HOTD solution.

Fixation

All of the HOTD treatment specimens showed partial fixation with a portion of the pulp not fixed, resulting in artifact. Four of the Ca(OH)₂ specimens showed very poor fixation and four showed partial fixation. Although this made evaluation very difficult, the investigators were still able to assess the other parameters.

Pulp Penetration

Investigators were able to see the small penetration by the dental bur into the pulp in 6 of the 8 Ca(OH)₂ specimens and all 8 of the HOTD solution specimens. In many of the specimens the mark of the depth of the bur penetration on the opposite side of the pulp chamber was also visible. It was clear that the assessment involved pulp, which had been penetrated and treated.

Treatment Material Contact with Pulp Tissue

In most of the specimens, material appearing to be ZOE was visible in the penetration tunnel made by the dental bur but in many we could actually see material inside the pulp of the tooth. In the Ca(OH)₂ specimens it was not possible to distinguish Ca(OH)₂ from ZOE. Material was in 4 (50%) of the pulps. In the HOTD solution specimens, material (presumably ZOE) was into the pulp tissue in 8 (100%) of the pulps. This may have been due to lack of the solid Ca(OH)₂ material over the pulp exposure before the placement of ZOE, failing to close the pulpal penetration tunnel which could otherwise serve as a barrier to the movement of the ZOE into the pulp tissue.

Vitality of the Pulp

The most difficult parameter to assess due to the

problem with fixation was the vitality of the pulp. However, investigators used other cues and the overall appearance of the pulp tissue to determine the vitality.

One of the Ca(OH)₂ specimens and one of the HOTD treatment specimens were totally vital. The remaining (87.5%) of the HOTD treatment specimens were partially vital and none of the HOTD solution specimens were non-vital. Three (37.5%) of the Ca(OH)₂ specimens were non-vital and 4 (50%) were partially vital. All of the HOTD specimens were vital or partially vital; 50% of the Ca(OH)₂ were vital.

Presence of Odontoblasts

Transformation into odontoblasts of the stem cells in the pulp was noted in 2 (25%) of the HOTD treatment specimens and none of the Ca(OH)₂ specimens (Fig. 1A). Three of the HOTD treatment specimens, which did not have identifiable odontoblasts, showed new dentin formation. There had to be odontoblasts present and we assume the problem of fixation made it impossible to see the odontoblasts involved in the pulp formation.

New Dentin Formation

None of the Ca(OH)₂ specimens showed formation of new dentin while 5 (62.5%) of the HOTD treatment specimens did. In one of the HOTD treatment specimens it was significant in that the new dentin was not only forming within the pulp adjacent to the penetration point but was actually forming out of the pulp area and into the tunnel within the dentin formed by the dental bur (Fig. 1B).

Summary for 7-Day Specimens

There was a significant difference in the formation of new dentin in the HOTD treatment specimens (62.5%) versus none in the Ca(OH)₂ specimens. None of the Ca(OH)₂ specimens showed odontoblasts transformation in the pulp while 25% of the HOTD specimens did. A significant difference was seen in non-vital pulps with 37.5% of the Ca(OH)₂ specimens being non-vital while no HOTD treatment specimens were. All of the HOTD treatment specimens showed treatment or sealing

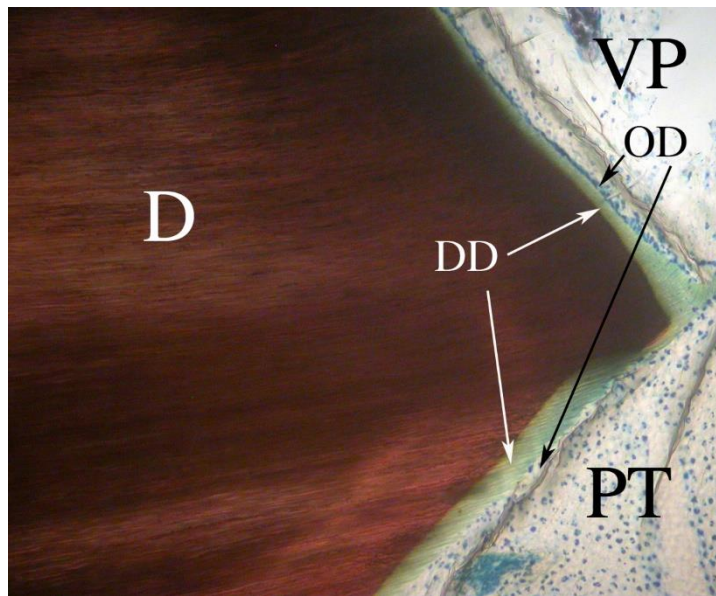


Fig. 1A. Seven-day HOTD treated pulp showing dentin (D), vital pulp tissue (VP), penetration tunnel (PT) with some inflammatory infiltrate, odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X200.

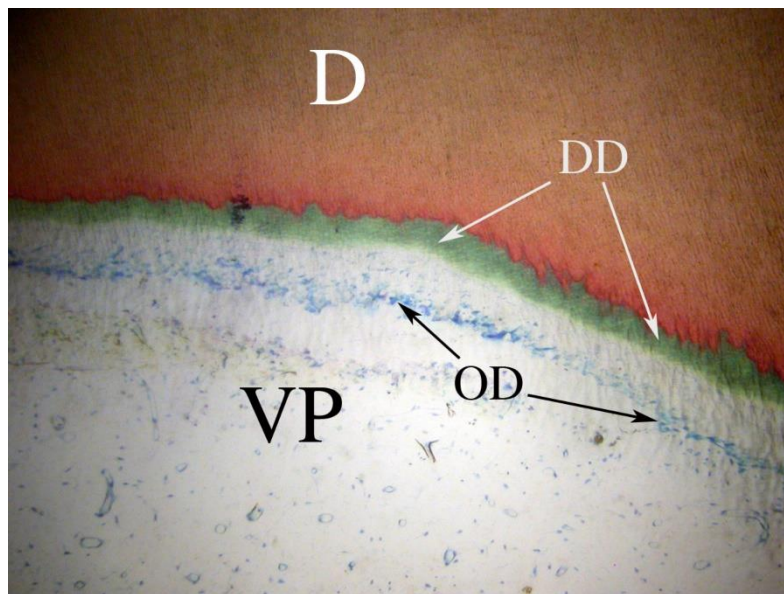


Fig. 1B. Seven-day HOTD treated pulp showing dentin (D), vital pulp tissue (VP), odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X200.

material actually within the pulp tissue while the $\text{Ca}(\text{OH})_2$ specimens showed 50%.

21-Day Specimens

Fifteen specimens were included in the 21-day study, eight were treated with $\text{Ca}(\text{OH})_2$ and seven with HOTD solution.

Fixation

The technique of specimen retrieval and fixation improved with this group of specimens compared to the 7-day specimens. Three of the $\text{Ca}(\text{OH})_2$ specimens showed poor fixation but the remainder of the $\text{Ca}(\text{OH})_2$ group and the entire HOTD treatment group showed good fixation. This made it much

easier to evaluate the other parameters.

Pulp Penetration

We were able to see the small penetration by the dental bur into the pulp in 6 of the 8 Ca(OH)₂ specimens and 6 of the 7 of the HOTD specimens. We were comfortable in the assessment knowing that we were truly seeing pulp that had been penetrated and treated.

Treatment Material Contact with Pulp Tissue

As in the 7-day specimens, in some of these specimens the treatment material was in the penetration tunnel made by the dental bur but in some we could actually see it inside the pulp of the tooth. In the Ca(OH)₂ specimens, the material was in the pulp in 4 (50%) of the pulps. In the HOTD solution specimens, the material was into the pulp tissue in 6 (86%) of the pulps (Fig. 2). As in the 7-day specimens, this may have been the result of having the solid Ca(OH)₂ preparation over the pulp exposure before the placement of ZOE to close the pulpal penetration tunnel, which could serve as a barrier to the movement of the ZOE into the pulp tissue.

Vitality of the Pulp

All (100%) of the HOTD treatment specimens had vital pulps. One had a significant amount of pulpal inflammation but the pulp was vital. Three (37.5%) of the Ca(OH)₂ specimens had non-vital pulps.

Presence of Odontoblasts

Transformation of stem cells in the pulp into odontoblasts was noted in 5 (71%) of the HOTD treatment specimens (Fig. 2) and 4 (50%) of the Ca(OH)₂ specimens (Fig. 3).

New Dentin Formation

The production of new dentin formation correlated with the visualization of odontoblasts in all of the specimens. New dentin formation was noted in 5 (71%) of the HOTD specimens (Fig.2) and 4 (50%) of the Ca(OH)₂ specimens (Fig 3).

Summary for 21-Day Specimens

There was a difference in the presence of odontoblasts and the formation of new dentin in the HOTD treatment specimens (71%) versus

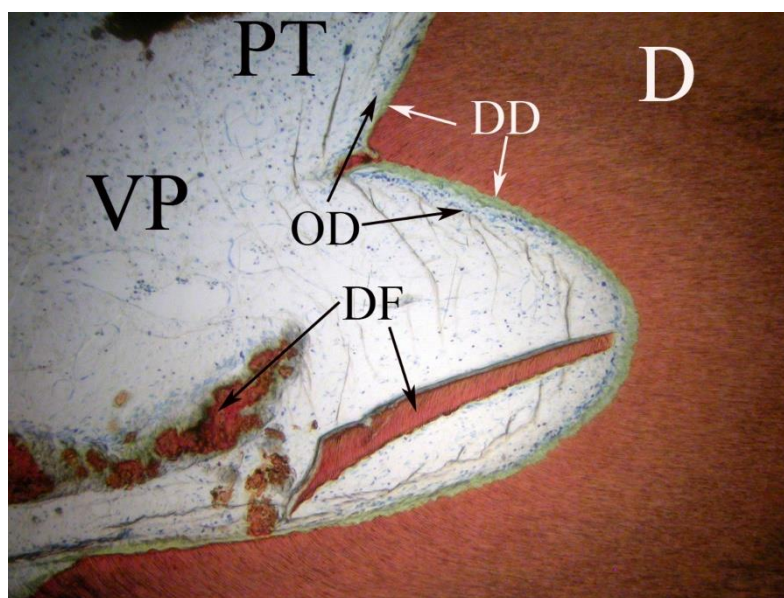


Fig. 2A. Twenty-one day Ca(OH)₂ treated pulp showing dentin (D), vital pulp tissue (VP), penetration tunnel (PT), dentin fragments pushed into the pulp from the penetration (DF), odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X100.

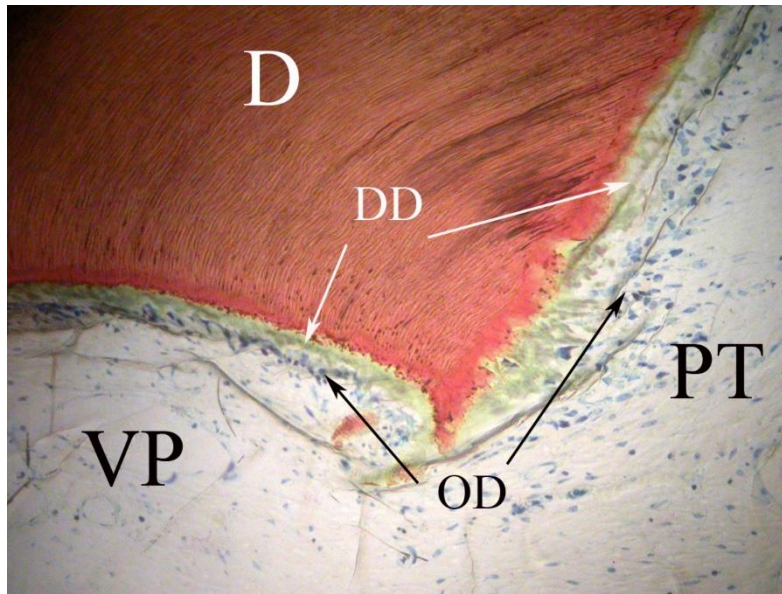


Fig. 2B. Twentyone-day $\text{Ca}(\text{OH})_2$ treated pulp showing dentin (D), vital pulp tissue (VP), penetration tunnel (PT), odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X200.

50% of the $\text{Ca}(\text{OH})_2$ specimens. A difference was seen in non-vital pulps with 62.5% of the $\text{Ca}(\text{OH})_2$ specimens being vital and 100% vitality of the HOTD treatment specimens. Eighty-six percent of the HOTD specimens showed treatment or sealing material actually within the pulp tissue while the $\text{Ca}(\text{OH})_2$ specimens showed 50%.

60-Day Specimens

Sixteen specimens were included in the 60-day study, 8 were treated with $\text{Ca}(\text{OH})_2$ and 8 with HOTD solution.

Fixation

The technique of specimen retrieval and fixation was the most successful of the study, with all teeth in both groups showing good fixation. This made it much easier to evaluate the other parameters.

Pulp Penetration

We were able to see the small penetration by the dental bur into the pulp in 100% of the $\text{Ca}(\text{OH})_2$

specimens and 7 of the 8 (87.5%) of the HOTD specimens. We were very comfortable in the assessment knowing that we were truly seeing pulp that had been penetrated and treated.

Treatment Material Contact with Pulp Tissue

In 7 of 8 (87.5%) of the pulps from both groups we could see the treatment or sealing material inside the pulp of the tooth. The material in the $\text{Ca}(\text{OH})_2$ treated pulps could have been $\text{Ca}(\text{OH})_2$ or ZOE.

Vitality of the Pulp

All of the HOTD treatment specimens and the $\text{Ca}(\text{OH})_2$ specimens had vital pulps.

Presence of Odontoblasts

Transformation of stem cells in the pulp into odontoblasts was noted in 100% of both the HOTD treatment specimens and the $\text{Ca}(\text{OH})_2$ specimens.

New Dentin Formation

The production of new dentin formation

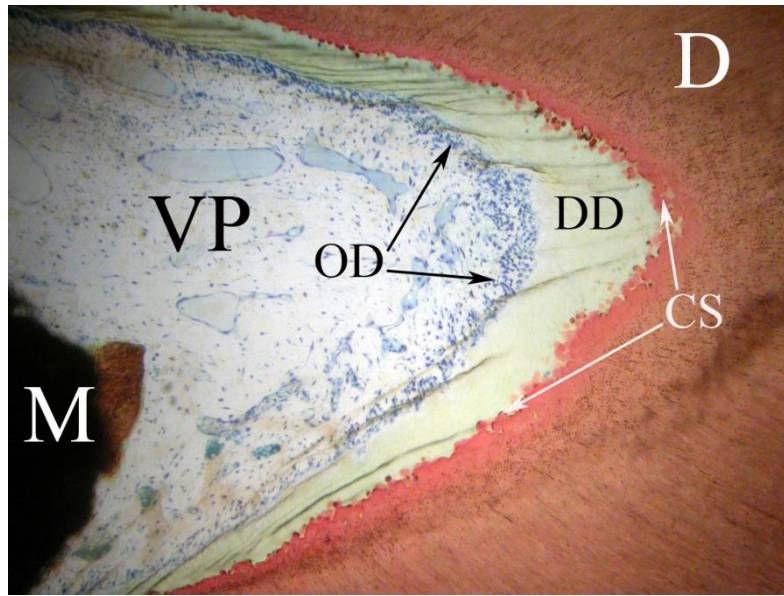


Fig. 3. Twentyone-day HOTD treated pulp showing dentin (D), vital pulp tissue (VP), odontoblasts (OD), sealing material (M), green-staining dentinoid (DD), and calcification of the dentinoid seen as calcospherites (CS). Stevenel's blue and Van Gieson's picro fuchsin X200.

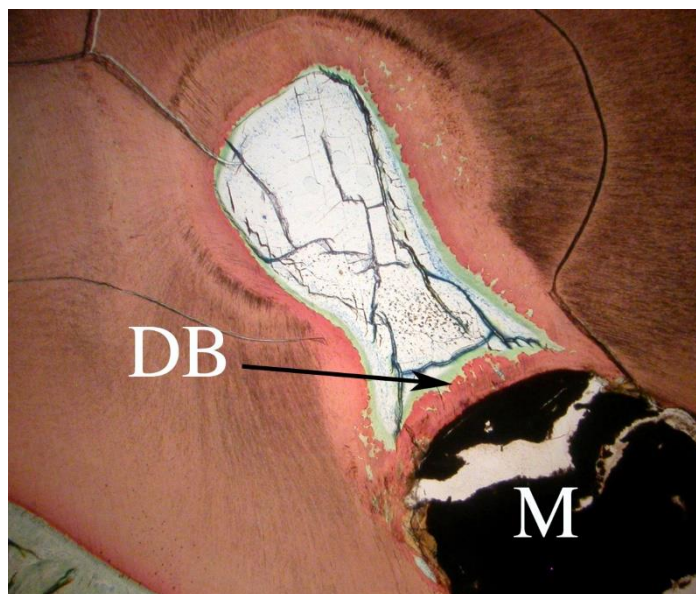


Fig. 4A. Sixty-day $\text{Ca}(\text{OH})_2$ treated pulp showing the nearly mature dentin bridge (DB) that has formed adjacent to the treatment and sealing materials (M) which were placed in the penetration tunnel. Stevenel's blue and Van Gieson's picro fuchsin X100.

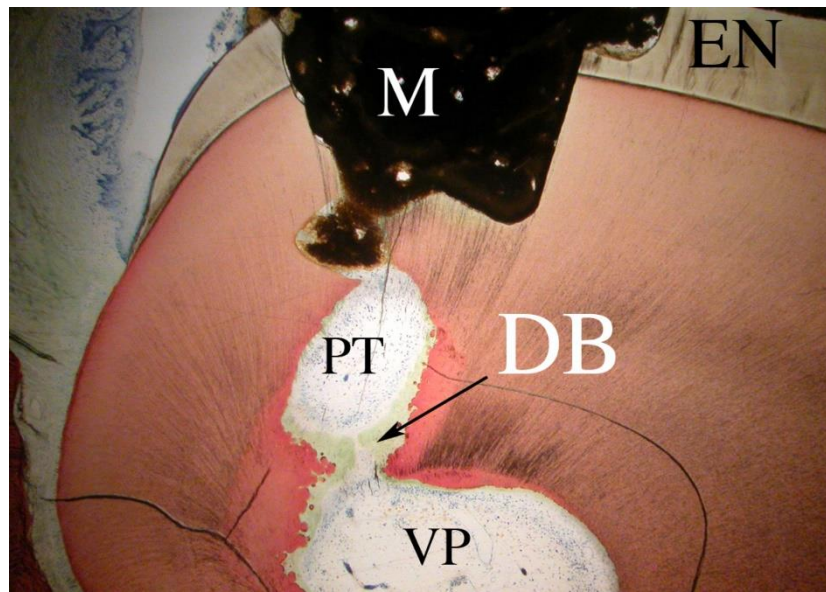


Fig. 4B. Sixty-day HOTD treated pulp showing the enamel (EN), sealing material (M) placed in the penetration tunnel (PT), vital pulp (VP), and the maturing dentin bridge (DB) closing the base of the penetration tunnel. Stevenel's blue and Van Gieson's picro fuchsin X100.

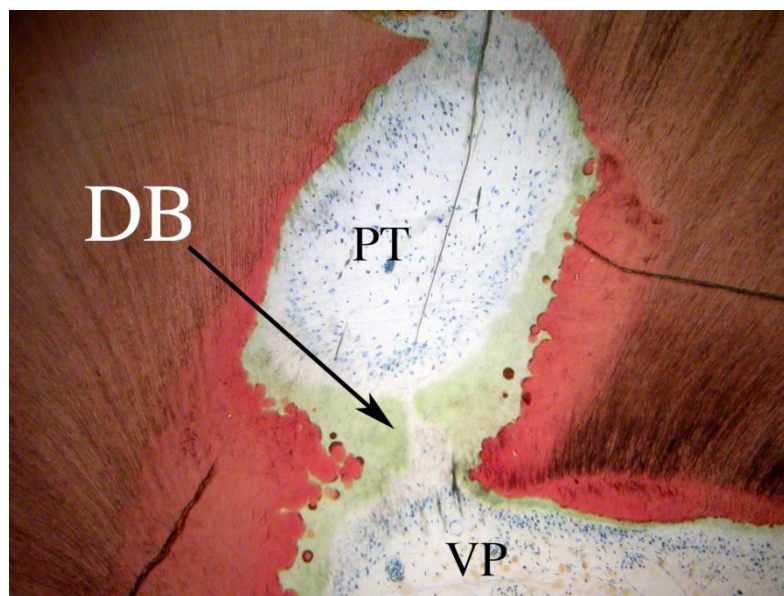


Fig 4C. Sixty-day HOTD treated pulp showing the penetration tunnel (PT), vital pulp (VP), and the maturing dentin bridge (DB) closing the base of the penetration tunnel. Stevenel's blue and Van Gieson's picro fuchsin X200.

correlated with the visualization of odontoblasts in all of the specimens. New dentin formation was noted in 100% of the HOTD treatment specimens and the $\text{Ca}(\text{OH})_2$ specimens. The production of new dentin was excellent for both materials including the production of dentin bridges closing the base of the penetration tunnel (Fig. 4).

Summary for 60-Day Specimens

There was virtually no difference between pulps of the HOTD treatment and $\text{Ca}(\text{OH})_2$ groups at 60 days. Fixation was excellent in all specimens. The histologic preparations were optimal. The pulps were vital in 100% of the teeth in both groups. Odontoblasts and excellent new dentin formation was seen in 100% of both groups.

DISCUSSION

Desiccating oral tissue decontaminant compared very favorably with $\text{Ca}(\text{OH})_2$ for direct pulp capping of vital dental pulp exposures, a therapeutic problem in dogs as a result of dental caries, in the attempt to remove dental caries or trauma to a tooth resulting in pulp tissue exposure. This study supported our hypothesis that the HOTD would be able to prevent acute inflammation leading to irreversible pulpitis, encourage odontoblast formation from stem cells and stimulate odontoblasts to form reparative dentin. When vitality of the pulp, presence of odontoblasts and new dentin formation were examined microscopically, for the seven-day pulps HOTD was significantly better than the long-standing standard treatment with $\text{Ca}(\text{OH})_2$. For the 21-day pulps HOTD was somewhat better than $\text{Ca}(\text{OH})_2$ and for the 60-day pulps it was absolutely equivalent to $\text{Ca}(\text{OH})_2$. Treatment of vital pulp exposures appear to be able to be treated with HOTD as well as with $\text{Ca}(\text{OH})_2$ with some apparent advantages at early post-treatment times. There was absolutely no harm seen in the pulps of the treated teeth in the analysis of any of the parameters analyzed associated with using HOTD compared to the standard $\text{Ca}(\text{OH})_2$ treatment.

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