Effect of Oleozon on Healing of Exposed Pulp Tissues

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Abstract: Objective: To compare the healing effect of Oleozon and calcium hydroxide on experimentally exposed pulp tissue. **Method:** Direct pulp exposures were conducted in nine dogs, i.e., three dogs were prepared for each experimental period of 7, 30 and 90 days. In each dog the upper and lower canines of the right side were capped with calcium hydroxide (Dycal), while those of the left side were capped with ozonated olive oil (Oleozon). After the observation periods, the teeth were prepared for histomorphological examination. **Results:** The tissues capped with Oleozon revealed inflammation with dilated blood vessels and hemorrhages at 7 days, a slight inflammatory response at 30 days, and the increase of collagen fibers and fibroblast with dilated blood vessels at 90 days. While, the tissues capped with calcium hydroxide exhibited medium degree of inflammation and necrosis adjacent to the exposure site at 7 days, remarkable absorption of necrotic tissues with few collagen fibers and fibroblast at 30 days, and a localized connective tissue capsule with depositions of reparative dentin at 90 days. **Conclusion:** Based upon the results and the limitations of the study, it was concluded that direct pulp capping with Oleozon induced less degrees of irritation to the dental pulp compared to that with Dycal. **Clinical significant:** The application of Oleozon paste for direct capping to exposed pulp could have a possibility to serve as a therapeutic method to enhance pulp tissue healing.
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Key words: Experimental pulp exposure, pulp capping, Calcium Hydroxide, Oleozon,histopathological examinations

1. Introduction

Preservation and maintenance of pulpal vitality has been a prime concern in operative dentistry. Historically, the placement of medicaments or materials against pulp exposures during caries excavation has been considered controversial, and instead conventional endodontic therapy has been recommended, (Langeland (1981); Tronstad and Mjör (1972)). The reluctance for direct pulp capping to pulp exposures in a carious field is based on unpredictable outcomes using traditional materials and treatment protocols. Moreover, compromised immune responses and impede cellular differentiation and recruitment, normal pulpal repair mechanisms may not function properly when bacterial by-products induce pulp inflammation. To date, researchers have never been identified a reliable non-absorbing bioactive pulp capping material that consistently stimulates cellular repair mechanisms, seals the dentin and promotes formation of a biologically stable reparative dentin bridge, Ward (2002).

Enhanced understandings in pulp physiology, caries progression, inflammatory mediators, and pulpal defence mechanisms have changed the clinical approach to caries removal and protocols for direct pulp capping, Hahn and Liewehr (2007). Several studies have demonstrated that the exposed pulp has the ability to heal when micro-leakage and bacterial contamination are prevented, Cox et al., (1987). A wide verity of materials and techniques for direct pulp capping have been used, including calcium hydroxide, hydrophilic resins, resin-modified glass ionomer cements, tri-calcium phosphates and, more recently, mineral trioxide aggregate(MTA), Heys et al.,(1981); Hørsted et al.,(1985); Matsuo et al.,(1996)and Auschill et al.,(2003) Success rates with direct pulp capping in a carious field have varied depending on the techniques and materials. In humans, success rates range from 30 to 85 percent in two- to 10-year retrospective studies, Barthel et al.,(2000); Baume and Holz (1981).

In fact, Calcium hydroxide, once considered the gold standard for pulp capping materials in which new materials should be tested, Farhad, A. and Mohammadi, (2005); Heys et al., (1980), but long-term studies have shown that the results were variable and somewhat unpredictable, Baume and Holz (1981); Hørsted et al., (1985); Barthel et al., (2000) and Auschill et al., (2003) .The material does not provide close adaptation to dentin, does not promote consistent odontoblast differentiation and has been shown to be cytotoxic in cell cultures; the resultant reparative dentin formation can be

characterized by tunnel defects, Schröder (1985); Andelin et al., (2003). Tunnel defects within dentin bridges may provide a pathway for the penetration of microorganisms to activate circulating immune cells, induce pulp irritation and produce subsequent dystrophic calcification Cox et al.,(1996).

Other innovative technical advances to halt the carious process and initiate the repair of potentially damaged tissue include the use of lasers, ozone technology and bioactive agents that induce and stimulate pulpal defenses, Moritz et al., (1998); Goldberg et al., (2003); Dähnhardt et al., (2006) . Ozone therapy is a modern noninvasive method of treatment. The word ozone was discovered by Schonbein in 1840 and comprises an allotropic variation of oxygen, Rilling (1983); Stopka (2003) . Ozone is an activated, trivalent form of oxygen. Ozone possesses strong antimicrobial activity, debriding effect, powerful oxidizing properties and can simulate angiogenesis, Sunnen (1988); Stopka (1999). Ozone is not only very powerful oxidizing agent that can kill a wide variety of viruses and bacteria, but also a very powerful non-chemical disinfectant, Seidler et al., (2008). Nowadays, ozone is employed as ozonated olive oil (Oleozon), which is widely used as a clinical therapeutic agent for wound healing Martinez-Sanchez et al., (2005). Ozonated materials, in which the ozone molecule stabilized as an ozonide between the double bonds of mono-saturated fatty acid, have the capacity to deliver nascent oxygen deep into the treated area without causing irritation. Under the influence of ozone, improved rheological properties, Verrazo et al., (1995), raised intracellular ATP, Oosting, et al., (1991) activated cellular metabolism , Shiratori et al., (1993) and expression of cytokines relevant to healing, especially Transforming Growth Factor (TGF-β1) were observed, Bocci et al., (1994).Ozone is currently used in dentistry as a possible antiseptic agent, and the effectiveness of ozone in the treatment of oral diseases is currently a subject of intensive investigations. Ozone has been used either in gaseous or in aqueous form for treatment of caries, in the root canal disinfection, and for enhancing epithelial wound healing, Filippi (2001); Nagayoshi et al., (2004); Baysan A, Lynch (2004). Flippi (2001)observed the accelerating effect of ozonized water on epithelial wound healing in the oral cavity. However, there is a lack of sound data on histological effect of ozone on oral tissues to minimize any possible side effects by controlling its applications , Bocci (2005).

Therefore, the purpose of this in vivo study was to compare the histomorphological characteristics of ozonated olive oil and calcium hydroxide cement when used as pulp capping agents in experimentally exposed dental pulp at different time intervals.

2. Materials and method:

2.1. Materials:

Calcium hydroxide containing product (Dycal, Dentsply/Caulk, U.S.A) and ozonated olive oil (Oleozon) were used as capping materials in this study. Ozonated olive oil paste was prepared through incorporation of ozone gas (O_3), generated by the Ozo-1m VTT unit (Ozomax, Qubba/Canada) with concentration of 70ug/ml (5%), into olive oil and was kept in a sterile sealed jar at -20C^o. Glass ionomer restorative material, Ketac Fil plus Aplicap **(**3M/ESPE, St Paul, MN, USA**)** was used as a restorative material.

2.2. Method:

Mixed breeds healthy dogs (n=9) between one and two years old were selected for the present study i.e., three dogs were used for each experimental period of 7, 30 and 90 days. After the quarantine period dogs were housed in individual cages with an area of 1.5m^2 and 1.5m high, provided with dry balanced diet, drinking bowels and cleansed daily. In each dog the right canines were capped with Dycal, while the left canines were capped with ozonated olive oil. Each dog was tranquilized by an IM injection of 10 mg/Kg of neurazine. General anesthesia will be performed by IV injection of Thiopental sodium in dose of 30mg/Kg body weight. The anesthetic effect occurred after 10-15 minutes and lasted for 30-60minutes. The selected teeth were cleaned and disinfected with Betadine. A dry field of operation was maintained by cheek retraction and placement of cotton rolls in the muco-buccal fold during operative procedure. Oval shaped class V cavities were prepared at 2 mm away from the gingival margin. The average dimensions of the cavities were 4mm in the longitudinal direction and 2mm mesiodistally. The cavities were prepared with a sterile #3 rounded bur using a low-speed engine with 25,000 – 30,000 r.p.m. under sterile physiological saline spray. The cavities were rinsed with sterile saline and dried with small cotton pellets. Pulp exposure was performed in the center of the pulp floor by means of #1 round bur under water coolant. Homeostasis was performed by irrigation with sterile saline and cotton pellet before placing the capping materials.

The application of Dycal was carried out according to the manufacturer's instruction. Equal amounts of the base and catalyst were mixed homogenously and placed gently over the exposure site using a #1 insertion spatula. They were served as a positive control group. For the corresponding pulp exposures on the left jaws, a thin layer of Oleozon was applied to the cavity using a small plastic instrument. It was spread evenly throughout the length of the exposed pulp in an incisogingival direction. Then, the cavities were restored with glass

ionomer restorative material. The animals were observed until recovery, and then every dog was transmitted to the cage after it regained its complete consciousness.

After observation period of 7, 30 and 90 days, the dogs were sacrificed by bilateral perfusion of formalin through cannula in common carotid arteries. The jaws were immediately dissected free, and the teeth were separated from the jaws by the use of handsaw. The apical root third of each tooth was amputated and immediately placed in individual bottles of 10 percent neutral-buffered formalin for 48 to 72 hours to allow proper fixation of pulp tissue. After fixation, they were placed in gauze bag tied with a string, which had been dipped in melted Paraffin .The bag was suspended in large quantity of decalcifying solution consisting of 5 percent aqueous solution of nitric acid for 1 to 4 days. The decalcifying agent was changed daily to maintain its effectiveness. The teeth were stored in decalcifying solution until obtaining certain degree of softening, which was checked by needle penetration. When they reached to required softening stage, every trace of decalcifying solution was removed by rinsing in running water for 24 hours, and then neutralized in 10 percent formalin. The additional irrigation was completed in running water for 24 to 48 hours. Dehydration was carried out in ascending grades of ethyl alcohol, and the specimens were then cleared in Xylene and embedded in Paraffin wax. Bucco-lingual cross sections of 7 microns were serially prepared in each cavity and stained using hematoxylin and eosin staining. The samples were evaluated under light microscopy to detect morphological alterations for each material and time period. The changes in pulp tissues were assessed using the Negm scale as weak, medium and considerable degree of inflammation.

The status of the odontoblastic layer was determined according to Qvist & Qvist (1977) with the following indexes: 0 as no reduction, 1 as insignificant reduction, and, 2 as medium to significant reduction.

The quantity of reparative dentin was evaluated by the method of Stanley on a 3−degree scale: 0 as no reparative dentin, ½ as initial formation of reparative dentin, and 1 as presence of a significant amount of reparative dentin.

3.Results:

3.1.Histomorphological analysis of the samples capped with Oleozon

The microscopic examination of the serial sections of the teeth showed vital pulp in all teeth. After 7 days, direct pulp capping with Oleozon revealed a considerable degree of inflammation with dilated blood vessels and hemorrhages. Significant reduction of the odontoblasts was detected Figure 1. However after 30 days, the histomorphological image changed drastically. A weak inflammatory reaction according to Negm, a well-organized layer of odontoblastoid cells, absence of hemorrhage, and persistence cellular infiltration were observed in Figure 2. After 90 days, increased collagen fibers and fibroblast with dilated blood vessels were detected in Figure 3.

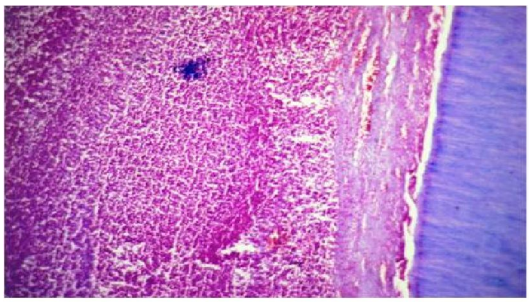

Figure 1 Morphological changes in canine dental pulp 7 days following direct capping with Oleozon

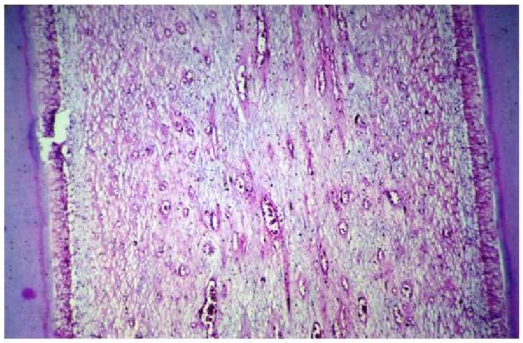

Figure 2 Morphological changes in canine dental pulp 30 days following direct capping with Oleozon

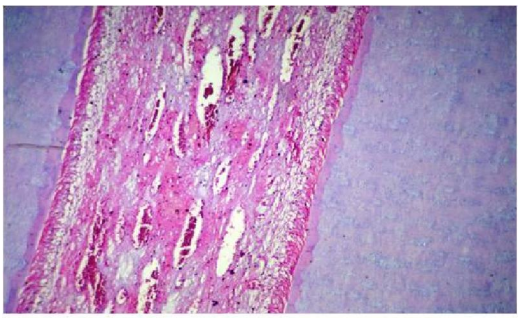

Figure 3 Morphological changes in canine dental pulp 90 days following direct capping with Oleozon

3.2.Histomorphological analysis of the samples capped with Dycal

The histomorphological changes of exposed pulp contact with Dycal revealed medium degree of inflammation, mononuclear cellular infiltration and necrosis at site of exposure at 7 days. The odontoblastic layer shows index 2 by Qvist adjacent to the damaged area. No reparative dentin was detected. The predentin was very thin and almost

absent near the exposed cavity. The rest of the pulp appeared full of congested and dilated blood vessels with extravasations particularly at the central portion of pulp cavity shown in Figure 4. 30 days specimens demonstrated Qvist index 1 and Stanley index 0. A weak inflammatory reaction, remarkable absorption of necrotic tissues at the exposure site with few collagen fibers and fibroblast were detected. The rest of the pulp appears nearly normal. There was an evidence of thin layer of dentin bridge formation shown in Figure 5, which was not always complete. After 90 days, a localized connective tissue capsule was detected between area with inflammatory reaction and healthy pulp tissues. Fibroblasts, small blood vessels and peripherally cited collagen fibers were detected inside this restrictive capsule. There was a prolific deposition of reparative dentin, as thin collar of osteodentin, with irregular and immature morphology as shown in Figure 6.

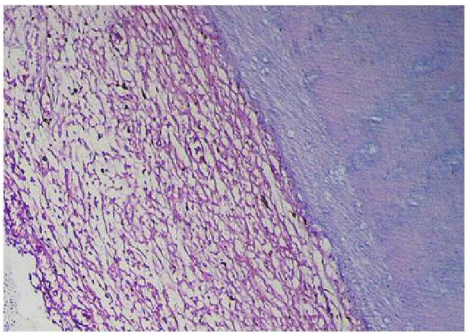

Figure 5 Morphological changes in canine dental pulp 30 days following direct capping with Calcium Hydroxide.

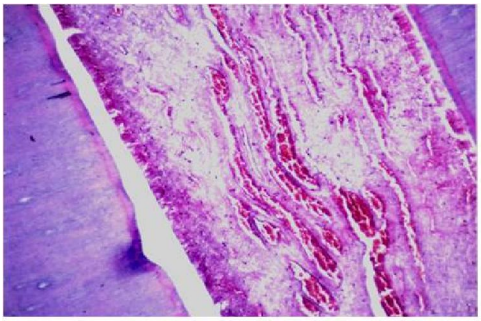

Figure 4 Morphological changes in canine dental pulp 7 days following direct capping with Calcium Hydroxide.

Discussion

Pulp capping comprises adequate protection of exposed dental pulp by the applications of medicaments and restorative material. This technique aims to preserve the vitality of pulp tissue, thus avoiding more invasive endodontic treatment. Although pulp exposure frequently occurs by caries process in which there is inflammatory reaction, the selection of healthy vital teeth for this study has the benefit of standardization and can be regarded as acceptable method in determining the effects of tested materials. In judging the efficacy of pulp capping materials, it is important to evaluate the presence of inflammatory reaction, necrosis and calcified bridge formation.

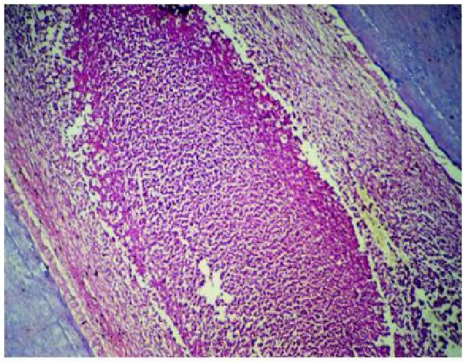

Figure 6 Morphological changes in canine dental pulp 90 days following direct capping with Calcium Hydroxide.

Based on the properties of ozone, the present study was designed to test ozonated olive oil paste as a direct pulp capping material in dogs' teeth. Ozone is very unstable gas. In air, ozone has longevity of only a few minutes; while in water, ozone lasts for a few days. However when ozone dissolved in an oil base such as 100% pure olive oil, ozone has a life span measured in months to years. The amount of oxygen and ozone saturation level is exponentially greater in solid paste than in liquid oil product. Also, the liquid does not have the long time release which ozonated olive oil paste does, Sechi et al., (2001). Different ozonized solutions such as vegetable oils have been used successfully against different infections. Vegetable oils are natural products derived from plant origin consisting of ester mixtures of glycerol with chains of fatty acid contain 14 to 20 carbon atoms with different degree of unsaturation, Ferrariet al.,(2004). The unsaturated triglycerides in vegetable oils were responsible for its favorable properties. There are different vegetable oils derived from various sources. Knowledge of their composition provides insight into their physical and chemical properties. Ozonized olive oil (Oleozon) was widely used for its therapeutic and antimicrobial effect against bacteria, fungi and virus, Hammeret al., (1999). The olive oil is obtained from olive tree and presents a high proportion of oleic acid (65-85%). It is characterized by high ratio of polyunsaturated fatty acids, such as the monounsaturated oleic acid and the polyunsaturated linoleic acid, Gertz et al.,(2000). Ozone chemically reacts with carbon-carbon double bonds present in unsaturated fatty acids producing long complex molecules called 'ozonoids'. These ozonoids are effective antimicrobial agents, and

stimulate the reparative and regenerative pathways at cellular level. Hydrolysis of ozonized oil will generate different products such as hydroperoxides, acetones, peroxides, and aldehydes and these compounds could be also responsible for the wide biological activity of ozonized olive oil, Siqueira et al.,(2000); Valacchi et al.,(2005). Furthermore, ozonated olive oil remains stable for 2years when stored at 4°C Valacchi et al.,(2005). The safety of Oleozon was discussed by Gundarova et al. (1996) and Alvarez et al. (1997) The present study is the first study that uses ozonated olive oil as direct pulp capping material. The histomorphological results of Oleozon application to the pulp showed a diffused involvement of the inflammation. It was reported that ozone exposure is associated with activation of growth factors, which are important to regulate inflammatory reactions and consequently the process of wound healing, Hørsted et al.,(1985);Pierce et al.,(1988); Werner and Grose (2003);Lim et al.,(2006). The increase of collagen fibers and fibroblasts indicated that ozone might act on healing directly or indirectly through collagen synthesis and fibroblast proliferation. Fibroblasts have been known to play an important role in reepithelization, synthesis of collagen fiber, extracellular matrix regeneration, and for the release of growth factors, Bocci(1994). The deposition of collagen by fibroblasts during the inflammatory process is a key event for human pulp repair, Barkhordar et al.,(2002); Chan et al.,(2005). Kim et al (2009) stated that the increased expression of platelet derived growth factor (PDGF) and transforming growth factor-β (TGF- β) were correlated with increased collagen fibers and fibroblast proliferation. TGF-β1 has a direct influence on cell proliferation, angiogenesis, chemotaxis (fibroblasts and monocytes), synthesis of extracellular matrix and collagen synthesis, Pierce et al.,(1988);Jazwa et al.,(2006).Also there was increase in vascularity in the ozone group. This was explained by Kim et al (2009) in which the increased vascularity might be due to the increased expression of vascular endothelial growth factors (VEGF) by ozonation, which is the main cytokine of vascularization in the late phase of healing. Bocci et al (1994) stated that the improved delivery of O_2 and release of growth factors appear beneficial in accelerating healing of wounds. Therefore, the treatment of the pulp with ozonated olive oil probably works by promoting collagen synthesis, fibroblast proliferation, and improvement of circulation. Taken together, we can infer that ozone application may be regarded as a therapeutic method to enhance pulp tissue healing.

On the other hand, the results obtained with calcium hydroxide are similar to the findings of others in direct pulp capping, Stanly and Lundy (1972);Eskandarizadeh et al.,(2006); Modena et al.,(2009). The superficial necrotic layer (cauterization zone) could be due to the chemical injury caused by hydroxyl ions, which present highly alkaline pH, yielding to an initial destructive effect , Glass and Zander (1949); Holland(1971). It has been reported that the high alkaline pH can solubilize and release some proteins and growth factors from dentin , Modena et al.,(2009). It was evident that, calcium hydroxide was able to enhance the pulp to perform its normal functions including deposition of secondary dentin. This was evident especially after 90 days test period. Dentin bridge formation occurred mainly from the periphery of exposure site, as its thickness at the periphery was greater than the thickness toward the prepared cavity. This may indicate its formation from the residual dentin chip at the wound surface, Kitasako et al., (2000); Parirokh et al.,(2005). These events may be responsible for pulp healing and hard tissue barrier formation.

So far, ozonated olive oil has the capacity to serve as a therapeutic agent. This highlights the importance of conducting further researches on ozone, which has encouraging expectation for further clinical applications.

Conclusions

Based upon the results and the limitations of the study, it was concluded that direct pulp capping with Oleozon induced less degrees of irritation to the dental pulp compared to that with Dycal. The application of Oleozon paste for direct capping of exposed pulp could have a possibility to serve as a therapeutic method to enhance pulp tissue healing.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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