



## Comparative Histological Study of Reparative Dentin Formation of Calcium Hydroxide and Zeolite in Pulpotomized Teeth (Histological Study)

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### KEYWORDS

Calcium hydroxide, Zeolite,  
Dentin bridge, Pulpotomy, Dogs.

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### ABSTRACT

**Aim:** Zeolites induce the proliferation and differentiation of cells of the osteoblast lineage. Application of zeolite in pulpotomized young permanent teeth had not been investigated. The objective of this study was to investigate histologically the capability of zeolite to induce dentin bridge formation and compare it with calcium hydroxide. **Subjects and methods:** Four dogs aged 12-24 months were included in the present study. In each dog, pulpotomy procedures were carried out in four premolars teeth in each quadrant and restored with one of the experimental materials in a way that teeth in each quadrant restored with one material differ from that of the contralateral quadrant. Specimens were harvested on the sixth week after operation and twelfth week after operative procedures, prepared and examined histologically by H&E (haematoxylin and eosin) stain, there were wide histological differences between the groups of this study along the different intervals of the study. **Results:** The result of the present study revealed inflammatory response of pulp tissue to both tested materials with various degrees that were subsided by time only in calcium hydroxide group. Calcium hydroxide has a powerful agent for regenerative effect on pulp tissue with dentin bridge formation. On the contrary; pulp degeneration and necrosis were the end results of pulpotomy with zeolite material. **Conclusion:** Calcium hydroxide could induce dentin bridge formation while zeolite failed.

### INTRODUCTION

The treatment of severely decayed and pulpally involved permanent teeth in the child or adolescent has long presented a challenge for ideal conventional root canal filling<sup>[1,2]</sup>. Pulpotomy may be an alternative to extraction for those patients who are unable to afford conventional endodontic therapy<sup>[3,4]</sup>. Many pharmacotherapeutic agents have been utilized for capping the radicular pulp after pulpotomy. However, the success rates have varied with the agent used and with the various techniques<sup>[5-7]</sup>.

Calcium hydroxide is extensively investigated early as a pulp capping agent to stimulate pulpal healing and dentin bridge formation, repair root fractures, perforations, open apices and root resorption where it induce apical root closure in affected mature teeth as well as in immature teeth<sup>[8]</sup>. Calcium hydroxide is still the primary standard treatment against other tested materials<sup>[9]</sup>. Some investigators asserted that alternatives to calcium hydroxide should be considered because of the clinical dissolution of calcium hydroxide, the recurrence of pulp inflammation and necrosis, and the presence of tunnel defects in dentin bridges formed against calcium hydroxide that increases its permeability<sup>[10]</sup>.

Zeolites are microporous, aluminosilicate minerals possess biological activities, antioxidative and immunostimulatory effects. Moreover it has been used as an adjuvant to anticancer therapy<sup>[11]</sup>.

Zeolites induce the proliferation and differentiation of cells of the osteoblast lineage<sup>[12]</sup>. When mixed with glass ionomer cement powder and root canal filling materials, they will act as an antibacterial agent<sup>[13]</sup>. In order of this, zeolite investigated as a possible alternative to calcium hydroxide in pulpotomy of young permanent teeth.

## MATERIALS AND METHODS

This study was carried out on four dogs aged 12-24 month, tow dogs for each period. In each dog, pulpotomy procedures were carried out in four premolars teeth in each quadrant. The animals were kept for 2 weeks preoperatively in the animal house of the faculty of veterinary South Valley University under standardized conditions.

Food was restricted 12 hours pre-operatively while water was restricted in the last 2 hours. Each dog was injected subcutaneously; 20 minutes before anesthesia, by atropine (Adwia, Egypt) at a dose of 0.04mg / kg body wt<sup>[14]</sup>. General anesthesia was obtained by intravenous injection of thiopental sodium (Epico, Egypt) at a dose of 3-5mg / kg body wt. A cannula, 18-20 gauges, was fixed in the radial

vein to be used for additional injection of anesthetic drugs if needed, intubation with a cuffed endotracheal tube. Special mouth gag was used to keep the mouth opened.

### Cavity preparation:

Class V cavities were prepared on the cervical third of the buccal surface of each tooth. Pulp exposure was performed in the middle of the cavity floor, the coronal pulp was removed.

### Experimental materials:

1. Zeolite mixed with normal saline.
2. Calcium hydroxide powder (ADWIC) mixed with normal saline.

Each quadrant had one of the tested capping materials, in such a way that the premolars in right capped with Zeolite would always have the contralateral (left) capped with Calcium hydroxide. All the remaining part of the cavity in the crown of every tooth (tested and control) was filled with amalgam. (Non gamma II SDI). Two dogs were euthanized after 6 weeks and the other two dogs after 12 weeks post operatively. pulpotomized teeth were assessed histologically by H&E.

## RESULTS

The histological results of calcium hydroxide treated teeth after six weeks of pulp capping, showed areas of liquefactive necrosis with inflammatory cell infiltration. The exposure site was closed by granulation tissues rested on numerous blood vessels, the odontoblastic layer appears with its shape close to normal with palisading pattern and multipl mitotic. Newly formed dentin (active dentinogenesis) characharized by very thick layer of predentin demarcated from primary dentin by separating line. The core of the pulp appeared close to normal structures containing cells and fibers. Fig. (1)

The histological results of zeolite treated teeth after six weeks of pulp capping showed, degenerated zones in the superficial layer of the pulp close



to the exposure site, disorganized odontogenic zone with signs of degeneration odontoblastic vacuolization, no signs of predentin formation. The remaining pulp structure showing less cellularity and increased fibrosis (fibrotic degeneration) interspersed with inflammatory cells. Fig. (2)

The histological results of calcium hydroxide treated teeth after twelve weeks of pulp capping showed, reactive responses of the pulp to capping processes. The most important reaction noted in this periods is the formation of predentin at the exposure site making continuity of dentin. The odontoblastic layers appeared in normal pseudostratified, pal-lassading appearance with different mitotic figures

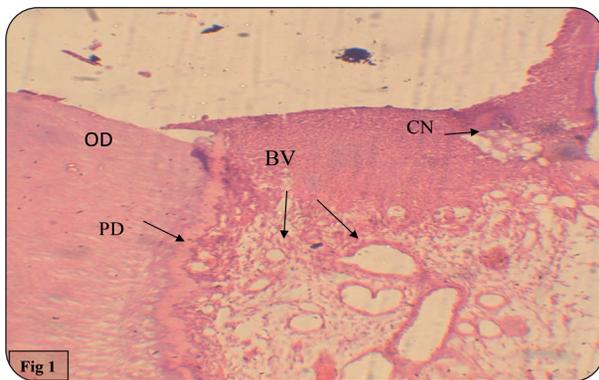


Fig. (1): Photomicrograph of Calcium hydroxide pulpotomy after six weeks. Showing (BV) multiple proliferated blood vessels and odontoblastic layer appears normal and appearance of (PD) predentin layer, (OD) old dentin and areas of inclusion bodies (CN) (coagulative necrosis). H&E stain (10 $\times$ ).

underneath the newly formed predentin (Dentin Bridge). The pulp tissues formed of their normal layers including odontoblastic layer, cell free zone, cell rich zone and pulp core. Fig. (3)

The histological results of zeolite treated teeth after six weeks of pulp capping showed, disappearance of odontogenic zone which was nearly occupied by a layer of inflammatory cells. There were no signs of odontoblasts proliferations, and /or predentin formation. The cells of the core of the pulp become separated from each other by collection of edematous fluid and decreased in their numbers as a sign of degeneration, the fibrous content decreased with less number of blood vessels. Fig. (4)

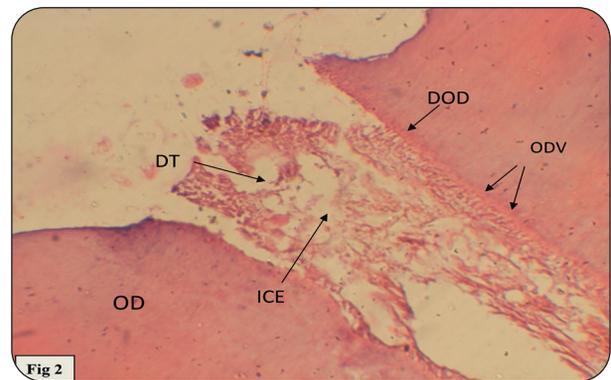


Fig. (2): Photomicrograph of zeolite pulpotomy after six weeks. Showing (DOD) disorganized odontoblasts, (ODV) odontoblasts vacuolation, (ICE) inter cellular edema, (DT) degenerative tissues, (OD) old dentin. . H&E stain (10 $\times$ ).



Fig. (3): Photomicrograph of Calcium hydroxide pulpotomy after twelve weeks. Showing (OD) Old dentin, (DB) dentin bridge, (P) Pulp and (OB) odontoblastic layer. H&E stain (10 $\times$ ).

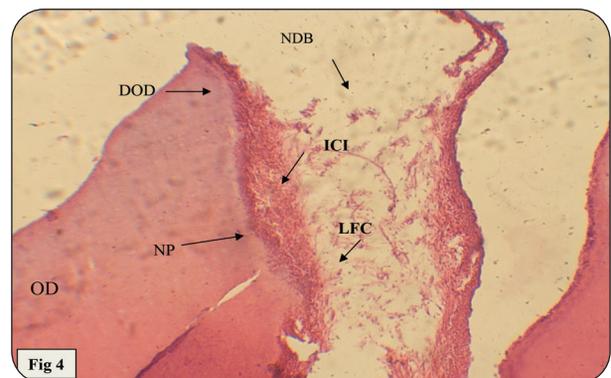


Fig. (4): Photomicrograph of zeolite pulpotomy after twelve weeks. Showing (ICI) Inflammatory cell infiltrate and (DOD) disappearance of odontoblastic layer, (LFC) less fibrous and cellular content (NP) no predentin, (NDB) no dentin bridge, (OD) old dentin. H&E stain (10 $\times$ ).

## DISCUSSION

Maintaining the vitality of dental pulp has been one of the fundamental concepts of dental treatment for a long time. If the pulp chamber is exposed, it is usually treated by direct capping or pulpotomy. The procedure relies on the ability of the dentin-pulp complex to repair dentin [15-19].

Calcium hydroxide is the most common material for capping of exposed pulp [20]. Due to its alkalinity; it has a bactericidal effect and stimulates the formation of reparative dentin. Unfortunately with some drawbacks like dystrophic calcification, discontinuous dentin bridge with the so called "tunnel defects" and internal root resorption [21].

The present study was designed to evaluate the effect of two different materials on pulp tissues, namely, calcium hydroxide and zeolite on the pulp tissue after pulpotomy in dogs' premolar teeth. Using H&E stain for histological examination.

The histological results of calcium hydroxide treated teeth after six weeks of pulpotomy showed, areas of liquefaction necrosis, Inflammatory cell infiltration mainly microphages and neutrophile around the degenerated tissues. Similar results obtained in other searches [14,22], they stated that small nodules resembling osteodentin outlined by a distinct layer of odontoblast-like cells could be seen forming in the pulp tissue along the wound margin.

The odontoblastic layer appears with its shape close to normal with pseudostratified appearance and multiple mitotic figures as a sign of regeneration interspersed by multiple proliferating blood vessels in between newly formed odontoblasts in different zones with pre-dentin formation, The newly formed dentin (active dentinogenesis), characterized by very thick layer of pre-dentin which demarcated from primary dentin by separating line. These results were close to the observation of other studies [14,23-25], they stated that, the increase in pre-dentin width could indicate a faster recovery of primary odontoblasts, with the beginning of tertiary dentine synthesis after the injury, caused by cavity preparation.

On the other hand zeolite treated teeth at the same period revealed presence of degenerated zones in the superficial layer of the pulp close to the exposure site. Disorganized, vaculated and degenerated odontoblasts with no signs of pre-dentin formation.

Histological examination of calcium hydroxide treated teeth after twelve weeks showed, reactive responses of the pulp to capping processes. The most important reaction noted in this periods is the formation of pre-dentin at the exposure site making continuity of dentin. The odontoblastic layers appeared in normal pseudostratified, palisading appearance with different mitotic figures underneath the newly formed pre-dentin (Dentin Bridge). Other researchers [25]. Obtained similar results at where dentin bridge formed. The pulp tissues formed of their normal layers including odontoblastic layer, cell free zone, cell rich zone and pulp core.

On the other hand histological observation of zeolite treated group at twelve weeks interval showed dramatic histological results. The pulp tissue failed to reorganize, the odontogenic zone disappeared and nearly occupied by a layer of inflammatory cells. There were no signs of odontoblasts proliferations, and /or pre-dentin formation. Neither pulp repair nor dentin bridge formation was observed.

In both periods evaluated in this study, no signs of migration and cell differentiation on the exposed area were observed in teeth capped with zeolite. The absence of primary odontoblasts and newly organized odontoblast-like cells result in the lack of dentin bridge formation. Similar pulpal responses have been described after direct pulp capping in human teeth and dog teeth using different capping agents [26].

## CONCLUSIONS

1. Calcium hydroxide remains the agent of choice for the capping of mechanically exposed pulps.
2. Calcium hydroxide has a powerful agent for regenerative effect on pulp tissue with dentin bridge formation.



3. The use of zeolite for pulpotomy in young permanent teeth, even after successful homeostasis, is questionable.

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## دراسة نسيجية مقارنة لتكوين عاجين الاسنان الاصلاحى بهيدروكسيد الكالسيوم والزيلوليت لاسنان منزوعة اللب جزئيا

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### الملخص :

**الهدف :** الزيلوليت هو مادة لها قدره على تكاثر وتمايز نسل الخلايا البانيه للعظام. استعمال الزيلوليت فى الاسنان الدائمه الشابه منزوعة اللب جزئيا لم يتم اختياره بعد. هدف هذه الدراسه هو اختبار قدرة الزيلوليت نسيجيا لاستحثاث تكوين جسر العاج ومقارنته مع هيدروكسيد الكالسيوم.

**المواد والطرق:** اربع من الكلاب بعمر 12-24 شهر قد شملوا فى الدراسه الحاليه. وتم اجراء نزع جزئى للب 4 ضواحك فى كل ربع من الفكين واستخدمت ماده واحده لكل ربع باستخدام تقنيه تقسيم الفم. تم جمع العينات بعد مرور 6 و 12 اسبوع بعد اجراء عمليات العلاج التحفظى وتم تحضير العينات وفحصها نسيجيا باستخدام صبغات الهيماتوكسيلين والايوسين وكان هناك اختلافات نسيجه واسعه ما بين المجموعتين فى الفترات البينيه المختلفه.

**النتائج:** اظهرت النتائج فى الدراسه الحاليه الاستجابه الالتهابيه لنسيج اللب للمادتين مع اختلاف الدرجات والتي قد هدأت مع الوقت فى مجموعه هيدروكسيد الكالسيوم . اكدت الدراسه ان هيدروكسيد الكالسيوم هو عامل قوى فى التأثير على اعاده تجديد انسجة اللب مع قدرته على استثارة اللب لتكوين جسر العاج وعلى النقيض فان الانحلال والتنخر هو النتجه النهائيه للب المنزوع جزئيا مع استخدام الزيلوليت.

**الاستنتاج:** نخلص هذه الدراسه الى ان هيدروكسيد الكالسيوم له قدره على تكويت جسر العاج بينما فشل الزيلوليت فى ذلك.

**الكلمات المفتاحية:** هيدروكسيد الكالسيوم، الزيلوليت ، جسر العاج، نزع لب السنه، كلاب.

