THE INFLAMMATORY RESPONSE OF PULP TISSUE AFTER DIFFERENT DIRECT PULP CAPPING MATERIALS AT DIFFERENT STORAGE TIME

Ali Yakout Dogheim AY1*, Walaa Mohamed Al Samolly2, Khalid Mohamed Noaman3, Khadiga Youssef Kawana4

ABSTRACT

Objective: Evaluate histologically and compare the inflammatory pulp response following direct pulp capping of mechanically exposed dogs’ teeth with: Ca(OH)2 base Dycal, UltraBlend Plus and TheraCal LC, at different time intervals. Subjects and methods. A total of 54 permanent teeth of six healthy dogs were used. They were randomly divided into three groups (n=18) according to pulp capping material used. Group I: Dycal, Group II: UltraBlend Plus (UBP) and Group III: TheraCal LC. All cavities were restored with Riva LC Resin Modified Glass Ionomer. Each group was subdivided into three subgroups according to storage time (n=6) at time intervals of 1 week, 1 month and 3 months. Control samples (3 teeth) were added representing normal untouched teeth for evaluating histological features of normal pulp. Paraffin sections were prepared stained with H&E then examined under light microscope for histologic evaluation of the inflammatory cell response. Results: Theracal specimens showed the lowest inflammatory score among the three capping materials in one week period followed by UBP and Dycal respectively. The inflammatory score by the end of third month improved for all materials. No inflammation was detected in Theracal specimens by the end of third month. Conclusions: Subsiding of inflammation over the study period is a sign of biological acceptance of the materials used in this investigation. Theracal is superior to Dycal and UBP for pulp capping of mechanically exposed human teeth, it can be used as an effective direct pulp capping material.

Keywords: Pulp capping, Theracal; UltraBlend, Dycal

INTRODUCTION

Pulp exposure could cause inflammation of the dental pulp (pulpitis), which may become irreversible, causing pain, pulp necrosis, and eventually may require an extraction or root canal treatment. Clinically, the treatment of pulp exposure includes a direct pulp capping to protect the dental pulp and preserve its vitality (1).

The overall aim of direct pulp capping is pulp healing. Several months after direct pulp capping, one of the following effects can be expected either regular pulp tissue without signs of inflammation with a constant layer of reparative dentine, chronically inflamed infiltrated pulp tissue with a permeable layer of reparative dentine interspersed with tunnel defects or highly inflamed pulp tissue with an imperfect, incomplete or missing layer of reparative dentine or a dense collagenous scar tissue in the area of pulp perforation (2).

Multiple pulp capping studies have demonstrated that pulp inflammation can be unrelated to bacterial presence, reinforcing the concept that certain materials applied directly to pulp tissue may elicit

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significant inflammatory response\(^{(3)}\). Many studies indicates that calcium hydroxide compounds are the
gold standard for pulp capping \(^{(4)}\). As a result of its
limitations, different formulations of Ca(OH)\(_2\) have
been developed and several dental adhesives have
been proposed in direct pulp capping. Hebling J et
al \(^{(5)}\) evaluated the cytotoxic effects of resin-based
light-cured liners like TheraCal and Ultradent
Plus (UBP), they concluded that Theracal was
least cytotoxic when compared to other materials.
As the choice between the wide variety of pulp
capping materials is somewhat difficult as well as
the difference of tissue response after direct pulp
capping; the objective of this study was to evaluate
histologically and compare the inflammatory pulp
response at different time intervals (1 week, 1
month and 3 months) following direct pulp capping
of mechanically exposed dogs’ teeth with: Ca(OH)\(_2\)
base Dycal, Ultradent Plus and TheraCal LC.

**SUBJECTS AND METHODS**

A total of 54 permanent teeth of six healthy
male mongrel dogs aged 18-24 months, and
weighing about 18-24 kg were used in the study.
The selected teeth were canine, second, third and
fourth premolars and, first molar, they were free
from caries, fractures and periodontal disease. The
fifty four teeth included in the study were randomly
divided into three groups (n=18) according to pulp
capping material used: Group I: Dycal, Group II:
Ultradent Plus and Group III: TheraCal LC.
All cavities were restored with Riva LC Resine
Modified Glass Ionomer.

Then each group was subdivided into three
subgroup according to storage time (n=6) at time
intervals of 1 week, 1 month and 3 months. Control
samples (3 teeth) were added representing normal
untouched teeth for evaluating histological features
of normal pulp.

Operations were done under general anesthesia
with intramuscular injection of 10 mg/kg of
Ketamine HCl and 1mg/kg of Xylazine. The
operation field, dogs’ teeth and oral cavity were
cleaned and disinfected with 0.2% Chlorhexidine
and rubber dam isolation was performed \(^{(6)}\).

A class V cavity was prepared 1mm away from
gingival margin on the buccal surface of the teeth
until pinkish shadow of the pulp was seen using a bur
number 330 in high-speed hand piece with copious
water spray. A number one sized round carbide
bur was used to drill in the middle of the cavity to
expose the pulp in a diameter not exceeding one
mm without impinging the pulp tissue \(^{(7)}\).

Pulp capping materials were applied according
to manufacturer instructions Dycal was mixed in
ratio 1:1 base paste to catalyst paste and placed
by burnisher in the cavity not more than 1mm in
thickness and left 2-6 min, UltraBlend Plus was
injected in the cavity at the exposure site and light
cured for 20 seconds and Theracal was injected in
the cavity at the exposure site and light cured for 20
seconds all materials was placed into the exposed
pulp without pushing it into the pulp tissue, materials
were randomly assigned into each quadrant.

Post-operative care of dogs included keeping
dogs under observation to assess presence or absence
of any infection, they were kept on soft diet and
receive a dose of a nonsteroidal anti-inflammatory
drug (Rimadyl Carprofen, Zoetis, USA) and have
demonstrated acceptable safety profiles to decrease
pain postoperatively \(^{(8)}\).

Two dogs were sacrificed with intravenous an-
esthetic overdose by injection of concentrated thi-
pental at the interval 1 week, 1 month and 3 months.

Jaw segments including the operated teeth and
parts of surrounding soft tissue were cut out of the
animal jaw to be prepared for histological exam-
nation. The specimens were fixed in 10% neural
buffered formalin, The specimens were decalcifi-
ced then in 8% trichloroacetic acid. The acid was
changed every three days for three weeks and
specimens were tested for complete decalcification,
then processed to paraffin blocks. Serial sections of
5 microns thickness were prepared and stained with H&E then examined under light microscope for histologic examination. The specimens were examined and evaluated histologically for inflammatory cell response following scoring system used by Nowicka et al. (9).

**TABLE (1) Nowicka scoring system.**

<table>
<thead>
<tr>
<th>Scores Inflammation intensity</th>
<th>1. Absent or very few inflammatory cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Mild (an average of &lt;10 inflammatory cells)</td>
<td></td>
</tr>
<tr>
<td>3. Moderate (an average of 10–25 inflammatory cells)</td>
<td></td>
</tr>
<tr>
<td>4. Severe (an average of &gt;25 inflammatory cells)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scores Inflammation extensity</th>
<th>1. Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Mild (inflammatory cells next to dentin bridge or area of pulp exposure only)</td>
<td></td>
</tr>
<tr>
<td>3. Moderate (inflammatory cells observed in one-third or more of the coronal pulp or in the midpulp)</td>
<td></td>
</tr>
<tr>
<td>4. Severe (all of the coronal pulp is infiltrated or necrotic)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dental pulp congestion</th>
<th>1. No congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Mild (enlarged blood vessels next to dentin bridge or pulp exposure)</td>
<td></td>
</tr>
<tr>
<td>3. Moderate (enlarged blood vessels in coronal pulp)</td>
<td></td>
</tr>
<tr>
<td>4. Severe (all coronal pulp is infiltrated with blood cells)</td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

As regards Dycal group: 66.7% of specimens showed severe inflammatory intensity and extensity together with dental pulp congestion “score 4” in 1 week storage time, while in 3 months storage time inflammatory intensity and extensity and dental pulp congestion were mild “score 2” in 66.7% of cases. So as a whole the inflammatory response of the pulp improves by time. This was shown by decreasing the inflammatory score from severe at 1 week storage time to moderate at 1 month and finally mild at 3 months storage time.

No statistically significant difference was found between the three studied storage time of Dycal groups as regards inflammatory intensity and extensity but for pulp congestion there was a statistical significant difference between the three studied storage time groups.

For UBP group: Specimens having direct pulp capping with UBP showed a moderate inflammatory intensity 66.7%, 83.3% extensity and dental pulp congestion “score 3” in 66.7% in 1 week storage time, while in 1 and 3 months storage time inflammatory intensity was mild “score 2” in 50% and 66.5% of cases respectively. The inflammatory extensity and dental pulp congestion were moderate in 1 month storage period in 66.7% and 83.3% of specimens respectively, both returned to mild in 3 months storage period in 66.7% of specimens. No statistical significant difference was present between the different time intervals in UBP group as regards inflammatory intensity, but statistically significant difference between different storage time of the study was detected in UBP as regards inflammatory extensity and congestion.

As regards inflammatory intensity and extensity in theracal: No significant statistic difference was found between groups. But for dental pulp congestion there was a statistically significant difference between the three time intervals.

Theracal showed the least congestion in 1 week and 1 month period and no congestion in 3 months period compared to Dycal and UBP.

When comparing the three materials: There was a statistically significant difference between theracal and both dycal and UBP as regards inflammatory intensity as well as pulp congestion after three months period

Regarding the effect of capping material on inflammatory score as function of evaluation periods, the data were collected and tabulated in table (2)
TABLE (2) Prevalence and test of significance for the effect of capping material on inflammatory score as function of evaluation periods.

<table>
<thead>
<tr>
<th></th>
<th>1 week</th>
<th>1 month</th>
<th>3 month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dycal (n = 6)</td>
<td>Ul. blend plus (n = 6)</td>
<td>Theracal (n = 6)</td>
</tr>
<tr>
<td></td>
<td>Dycal (n = 6)</td>
<td>Ul. blend plus (n = 6)</td>
<td>Theracal (n = 6)</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>----------------</td>
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<td>--------------</td>
</tr>
<tr>
<td>Inflammatory intensity score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>2 week</td>
<td>2</td>
<td>33.3</td>
<td>4</td>
</tr>
<tr>
<td>3 week</td>
<td>4</td>
<td>66.7</td>
<td>1</td>
</tr>
<tr>
<td><strong>MCp</strong></td>
<td>0.018*</td>
<td>0.368</td>
<td>0.041*</td>
</tr>
<tr>
<td>Inflammatory extensity score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
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<td></td>
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<tr>
<td>1 week</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>2 week</td>
<td>1</td>
<td>16.7</td>
<td>0</td>
</tr>
<tr>
<td>3 week</td>
<td>3</td>
<td>16.7</td>
<td>5</td>
</tr>
<tr>
<td>4 week</td>
<td>4</td>
<td>66.7</td>
<td>1</td>
</tr>
<tr>
<td><strong>MCp</strong></td>
<td>0.001*</td>
<td>0.194</td>
<td>0.142</td>
</tr>
<tr>
<td>Dental pulp congestion score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1 week</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>2 week</td>
<td>2</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>3 week</td>
<td>3</td>
<td>33.3</td>
<td>4</td>
</tr>
<tr>
<td>4 week</td>
<td>4</td>
<td>66.7</td>
<td>1</td>
</tr>
<tr>
<td><strong>MCp</strong></td>
<td>0.145</td>
<td>0.004*</td>
<td>0.001*</td>
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</tbody>
</table>

**MCp**: p value for Chi square test (Monte Carlo) for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

**Histological evaluation**

**One week (Fig 1,2)**

**Dycal group**: The histological picture showed loss of the normal architecture of the connective tissue stroma which was heavily infiltrated with inflammatory cells and dilated congested blood vessels. Ruptured blood capillaries with extravasated red blood cells and areas amorphous pale eosinophilic spaces (edema) were seen.

**UBP group**: Connective tissue stroma showed multiple vaculations, moderate inflammation with multiple polymorph nuclear leucocytes related to coagulative necrotic areas at the exposure site and dilated blood vessels were seen.

**TheraCal group**: The histological picture of pulp tissue exhibited alteration in architecture with moderate inflammatory cells and dilated blood vessels otherwise the connective. Tissue stroma showed no changes ..
One month (fig 3,4)

Dycal group: Connective tissue stroma showed moderate inflammation was seen with multiple polymorphonuclear leukocytes related to coagulative necrotic area at exposure site.

UBP group: The histological picture of pulp tissue exhibited variable degrees of inflammation reaction, either showing low grade cellular infiltrate with polymorph nuclear leukocytes in connective tissue or even coronal pulp involvement.

TheraCal group: Pulp appeared normal in structure with capillaries of different sizes and few scattered inflammatory cells in connective tissue stroma.

Three months (fig 5,6)

Dycal group: Dilated congested blood vessels and moderate inflammatory cellular infiltrate were seen in connective tissue stroma.

UBP group: Mild inflammatory cellular infiltration was seen, polymorph nuclear leukocytes were traced in the deep layers of the pulp connective tissue stroma.

TheraCal group: Normal connective tissue stroma with mild inflammatory cell infiltrate.

FIG (1) L.M. Dycal group one week showing severe inflammatory cell infiltration. (arrow) (H&E X 100)

FIG (2) L.M. UBP group one week showing; moderate inflammatory cells infiltration multiple polymorph nuclear leukocytes. (arrow) (H&E X 100)

FIG (3) Dycal one month showing moderate inflammation with multiple polymorph nuclear leukocytes. (H&E X 400).

FIG (4) L.M. Ultra Blend Plus one month showing moderate cell infiltration with polymorph nuclear leukocytes (arrow head), among disorganized pulp structure with dilated blood vessels. (arrow). (H&E X 100)
DISCUSSION

In the last decade, many experimental and clinical studies have been carried out to develop and test new dental materials endowed with safe biocompatibility and anti-infective properties. Direct pulp capping (DPC) has been frequently used to preserve vitality of dental pulp. The material used should provide an appropriate host response. This means that the tissues that come in contact with the materials should not show any toxic, irritating, inflammatory, allergic, genotoxic or carcinogenic response.

Nowika et al. and Suzuki et al. stated that in vivo studies are required to understand pulp response when dental materials are used for direct pulp capping.

Negma et al. and Matsuo et al. examined the length of time necessary for adequate postoperative follow up, and suggested that 3 months period was adequate for a tentative prognosis this was also chosen for our study.

Dog was the selected animal model as the mechanisms of induction and synthesis of dentin in this animal are the same as in human beings. The bleeding was controlled by rinsing the exposure site with sterile saline solution this method was also adapted by Al-shehbin et al.

Godoy and Murray concluded that bleeding control are most likely even more important to preserve pulp vitality than is the choice of a specific direct pulp capping agent, because of evidence that pulp healing is most compromised by hemorrhagic injury.

In order to assess the biological capability of the tested materials, histopathological evaluation was performed to determine the inflammatory reaction of pulp tissues over storage time of 1 week, 1 month, and 3 months to evaluate the pulp response to the three used capping materials: Dycal, Ultrablend plus and Theracal. The grading of their success was based on: pulp vitality and evaluation of pulp inflammatory score: the inflammatory intensity and extensity scores as well as pulp congestion.

In one week, the inflammatory intensity and extensity scores had statistically significant difference between all three materials used showing severe inflammatory intensity and extensity of dycal compared to ultrablend plus and theracal. Aenehechi et al. in 2003 studied the effect on pulp that was capped with calcium hydroxide cement and they were extracted after periods of 1 week, 2 months, 3 months, 4 months and 6 months. Histological evaluation demonstrated that the inflammatory response lasted for 6 months, starting...
with acute inflammation and ending in mild chronic inflammation. This was also proved by Stefanova et al.\(^\text{20}\) and was in concordance with our study.

Chen and Suh\(^\text{21}\) on the other hand, suggested that light curable resin modified calcium silicates, such as TheraCal LC, have less cytotoxicity and better or comparable clinical outcomes during the 1st week of DPC.

Current study showed that in one month, a reduction in the inflammatory response was evident in all groups. This represented a positive healing outcome. The results were statistically significant between the three materials after one month as regards inflammatory extensity and pulp congestion.

These findings are in agreement with Maden et al.\(^\text{22}\) and Modena, K.C.\(^\text{23}\) who described the inflammatory changes encountered with dycal and UBP and attributed them to be responsible for the pulp repair and hard tissue barrier formation. They attributed this to the high pH which induces a coagulation necrosis layer when in direct contact with pulp tissue.

The results of the present study revealed that TheraCal LC caused inflammation with varying intensities which was a normal response to a foreign material and subsided at 3 months interval. This may be attributed to excellent sealing properties which prevent microleakage and pulp inflammation by providing a predictable secondary barrier\(^\text{24}\).

In accordance with the previous studies, samples that were capped with Dycal showed less satisfactory results\(^\text{25,26}\).

The present histological analysis of direct capping with the three different materials confirms that the inflammation of the pulp is reversible. The few areas of inflammatory infiltrate observed and the small layer of pulp necrosis at one week, are probably caused by the preparation of the cavities or the penetration of the bur in the pulp, and it is not caused by different materials.

Most of the published studies Alquahtani et al.\(^\text{27}\) Goldberg et al.\(^\text{28}\) reported that the healing sequence starts with an initial moderate inflammatory process, and now there are evidences that inflammation is a prerequisite for tissue healing as a first step, followed by pulp regeneration, also described as pulp repair.

The presence of inflammation of the pulp until the longest storage time 3 months after capping although diminished yet it was a common feature in Dycal specimens; as stated by Parolia et al.\(^\text{4}\) and Nair et al.\(^\text{29}\).

Goldberg et al.\(^\text{28}\) stated that light curable resin based cements have been introduced to enable a better marginal seal and lesser dissolution; this may explain the reduction in inflammation at 3 months for UBP.

As regards TheraCal, Lee et al.\(^\text{25}\) considered the absence of polymorphonuclear leucocytes, a sign of acute inflammation, in favor of TheraCal LC which also correlates to clinical findings where no abscess formation was evident. TheraCal LC histologically appeared to be more pulpally kind as compared to other groups with very mild pulpal inflammation and minimal necrosis but there were no statistically significant differences between the groups in regard to pulpal inflammation (P=1.00)\(^\text{11}\).

Pulp tissue in the present study displayed intense to moderate inflammation with all the test materials at 1 weeks, then significant improvement occurred by 3 months, coinciding with the findings of Sadek H and Abou el Nasr H.\(^\text{30}\).

CONCLUSIONS

1. The subjacent pulp tissue play a role in pulp healing together with the pulp capping agents which help the process.

2. Calcium hydroxide based chemical cure cement (Dycal) evoked severe inflammatory response in the pulp. However it subsides over the study period which is a sign of biological acceptance.
3. Histological evaluation of pulp treated teeth using Theracal LC in animals have shown interesting and promising results that encouraged its use in human teeth.
4. Theracal and UBP share the indications and mode of action with Dycal but does not have its drawbacks, they can be used as an effective direct pulp capping material.

**RECOMMENDATIONS**

1. Although the short term results of Theracal LC are promising, the long term efficacy remains limited so, we need to study longer periods.
2. Studying the effect of direct capping on larger scales with larger sample size.

**REFERENCES**


