BERIPLAST, GELFIX AND CALCIUM HYDROXIDE AS DIRECT PULP CAPPING AGENTS-A HISTOPATHOLOGICAL STUDY ON DOG’S PULP

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Abstract
Pulp dressing potentials of two commercial surgical materials (Gelfix and Beriplast) were evaluated histologically in dogs, and compared with that of calcium hydroxide. Class V cavities were prepared in 60 fully developed permanent incisor teeth in five dogs. Following the standard intentional pulpal exposure procedure and wound treatment, capping materials were placed in direct contact with the exposed pulps and then the cavities were restored with ZOE cement and amalgam. The tissues were collected after 7, 30, 60 and 90 days. Histological sections were prepared and examined for Inflammation, necrosis, and bridge-like and tertiary dentine formations. Severe inflammatory responses to both tested materials were observed. In contrast, pulp response to calcium hydroxide over the test period at least, good, and showed a distinct healing capacity.

INTRODUCTION
Experimental and clinical trials suggest that pulp severed by exposure has, if properly treated, the intrinsic potential for carrying out repair or remodeling with a good prognosis (20). A number of materials have been proposed for direct pulp capping. Calcium hydroxide is the most commonly used pulp capping agent and pulp reaction to it has been well documented (13, 18, 25, 29). According to Schröder (29), the beneficial effect of calcium hydroxide is due to tissue necrosis produced by chemical effect. This firm necrosis of the pulp stimulates the underlying tissue to defend and repair by production of a hard-tissue barrier.

Attempts to maintain the vitality of dental pulps using a variety of dressings other than calcium hydroxide have been reported (13, 16, 17, 28, 42). To use a biological material that would lead to healing of the pulp wound, without irritating the tissue and yet inducing dentine formation, would certainly be ideal. Collagen has therefore been evaluated as a pulp dressing in several studies. These studies investigated the effect of collagen on normal and inflamed pulp in different animal models, although they reported conflicting results (5, 11, 23). Collagen has the potential to offer a wound insulation as well as matrix induction for the exposed pulp to repair by regeneration (6, 7). Fibrin tissue adhesives have also proved to be effective and valuable tools in dental surgical procedures (21, 26, 27). Extensive studies have demonstrated the safety and efficacy of collagen agents and fibrin tissue adhesives (1, 2, 19, 32, 36, 41).

The aim of this study was to evaluate the possible effect of two biological materials widely used in surgical procedures, a collagen based sponge (Gelfix) and a fibrin seal (Beriplast), on exposed intact pulp and to compare their potential use as direct pulp capping agents with that of an established material, calcium hydroxide.
MATERIALS AND METHODS

60 fully developed permanent incisor teeth of five young dogs, 3 to 4 years old, were used for this study. After premedication with Xylazine (2 mg per kg body weight, Rompun - Bayer, Germany), anaesthesia was induced (i.v. injection of Ketamine, 5 mg per kg body weight, Ketalar - Park Davis - USA) and maintained under 3% Halothane (Hoechst, Germany) following tracheal intubation. The teeth were thoroughly scrubbed with pumice and gauze soaked in 4% H2O2 solution. Teeth were isolated with rubber-dam and the operation site was cleaned with 5% tincture of iodine. Buccal Class V cavities were prepared by means of a No.2 round bur and a No.4 inverted cone carbide bur in an air turbine under adequate water spray. The pulps were exposed with sterile No.00 round burs, operated at low speed. The cavities were irrigated with saline solution and haemorrhage was arrested with sterile cotton pellets. The exposure sites were then immediately covered with one of the following dressings: a lyophilized bovine Achilles tendon collagen sponge (Gelfix, Eurosearch, Italy), a commercial fibrin seal (Beriplast, Behringwerke, Germany) and calcium hydroxide - normal saline paste (Calcium Hydroxide, Sultan, USA).

After the dressing materials were placed, the teeth were sealed with IRM and the remainder of the cavities restored with amalgam. All cavities in both upper and lower quadrants were dressed with calcium hydroxide at one side and Gelfix or Beriplast at the other. The animals were sacrificed and the tissues were collected at 7, 30, 60 and 90 days, fixed in 10% buffered formalin, and decalcified in 10% formic acid. After paraffin embedding, serial sections of the teeth, 8 microns in thickness, were cut in the bucco-lingual plane and stained with hematoxylin and eosin, or Brown and Brenn bacteria staining method (37). The evaluation was performed according to widely used differential diagnosis scoring criteria. (8, 9, 18, 33).

Inflammatory cell infiltration:
1. No or sparse (slight) leucocytes in contact with the original wound.
2. Localised (moderate) leucocyte infiltration subjacent to, or some distance from the original wound.
3. Massive (severe) leucocyte infiltration throughout the pulp.
Degenerative changes some distance from the original wound:
1. Collapsed or cavity-like pulp zone (liquefaction necrosis).
2. Large area of fibrin core organisation (coagulative necrosis).
3. Abscess form degeneration.
4. Atrophic (shrunken) pulp in association with heavy tertiary dentine deposition.

Bridge-like dentine formation:
1. In contact with dressing.
2. At some distance from dressing.
Tertiary dentine: Prominent or irregular newly formed dentine layer in presence of a well-developed odonto-blastic layer.
1. Mainly coronal.
2. Only radicular.
3. Both coronal and radicular.

RESULTS

The scores for the selected criteria are given in Table 1. As the teeth treated with Gelfix and Beriplast showed a fairly similar response pattern, in most cases below, they are both described together.

The original wound pattern:

At seven days, a vertically bulging structureless area under the exposure site, was seen in all specimens (for all materials) (Figs. 1, 2). The underlying coronal tissue was compressed in all directions. In teeth treated with calcium hydroxide the original wound exhibited an expanding vacuolar, blister-like appearance (Fig. 1A). However in teeth dressed with Gelfix and Beriplast, a chronic inflammation at mid-coronal position was organised in the pulp tissue. The cavity was partly filled with tissue or material debris (Fig. 2A).

Inflammation:

In all teeth dressed with calcium hydroxide, moder-
ate leucocyte infiltration was present in the vicinity of the original wound at 7 days, and also some distance from the exposure site beneath the newly formed dentine bridge at 30, 60, 90 days. Inflammation was acute at 7 days (Fig. 1A). However at other time intervals, a moderate chronic zone consisting of fibrotic organisation with scattered mononuclear leucocytes was present in the remaining pulp (Fig. 1B, 3A 3B). Inflammation was severe however, and predominantly chronic under both of the tested materials. Both the coronal and radicular pulp were involved. Many transitional chronic inflammatory stages were overlapping from the 7-day observation period onward (Fig. 2,4,5). Only in two specimens of the 7-day Gelfix group and one specimen of the 7-day Beriplast group was the cell infiltration moderate. At 30 and 60 days massive abscess formations were also present throughout the radicular pulp (Fig. 4C, 5B).

Degenerative changes:

At 30, 60 and 90 days, in the calcium hydroxide teeth, the coronal pulp had collapsed due to softening. The coronal part of the pulp cavity had emptied, and only some debris was seen in contact with the dentine walls. An atrophic evolution was also seen in the radicular pulp portion under the dentine bridge in association with a moderate chronic inflammation and heavy tertiary dentine deposition (Figs. 1B, 3A, 3B). In contrast, in the Gelfix and Beriplast dressed teeth, coronal necrotic degeneration was incomplete, and had spread into the radicular portion. Coagulative and softening necrosis stages were found to be overlapping in a large zone some distance from the original wound (Fig. 2, 4, 5).

Bridge-like dentine formation:

No barrier-like deposit was seen in any specimens adjacent to the exposure site. Only occasionally was some bulky tertiary dentine layer noticed on the edges of the perforation (Fig. 2C). However, at 30, 60 and 90 days, in teeth dressed with calcium hydroxide, a well-formed dentine layer was found far from the exposure site, and overlaying the remaining pulp trunk beneath the fully necrotic coronal pulp (Fig. 1B, 3A, 3B). On the radicular surface of this bridge, detached from the coronal and radicular pulp portions, a neat odontoblastic layer was extended in line with the peripheral pulp. As with calcium hydroxide, pulp appeared also hard tissue to respond near that same zone with both Gelfix and Beriplast, however such responses did not give rise to a complete bridge-like layer, and remained abortive (Fig. 2B).

Tertiary dentine layer:

A prominent or irregular dentine layer and a well-developed underlying odontoblastic layer were present in the calcium hydroxide dressed group at 30, 60 and 90 days (Fig. 1B, 3A, 3B), and only at 30 days in Gelfix and Beriplast groups along with chronic inflammation (Fig. 2C). In both the tested material groups, at 60 and 90 days, only sparse living odontoblastic layers remained.

DISCUSSION

Calcium hydroxide has long been the pulp capping agent of choice, and many reports confirming its hard tissue barrier forming repair potential have been published (12, 13, 14, 25). In the present study, at a post-operative period of 30 days, complete dentine bridges were only present with the calcium hydroxide dressing, only for a small distance below the exposure site. This finding is in agreement with those of some previous studies (13, 14, 18). Heya et al. (12) reported that Pulpdent Multi-Cal stimulated hard tissue bridge formation, but that its high alkalinity prevented tissue reorganisation and bridge formation at the material interface.

Dentine bridges formed at a short distance near the exposure sites in pulps treated with pure calcium hydroxide mixed with saline solution, which had a pH of about 12 (38). This was also reported by Heya et al. (12). The finding of dentine bridge formation after a 30-day period is not consistent with Pereira's (24) report of atypical calcified tissue only after 120 days in calcium hydroxide dressed dog's pulp. However, many
other authors have reported the presence of a calcified bridge after an approximately 30-day period (13, 15).

Watts and Paterson (40) regard the dentine bridge as a good prognostic indicator because it demonstrates the continued function of the odontoblasts near the site of injury. Odontoblasts are delicate cells (40), and the same authors (39) have suggested that their ability to function is an indicator of the state of the pulp. However, in spite of bridge formation, the remainder of the pulp may remain chronically inflamed (31). In our study, however, dentine bridge formation was observed with the calcium hydroxide-dressed teeth. The pulps of these teeth showed a temporary healing pattern through some distinct morphological stages and a moderate chronic inflammation persisted in the pulp underlying the dentine bridge from the 30-day observation period onwards, which did not subside even after 90 days. This finding is in agreement with that of Pereira (24), who reported the persistence of inflammatory cells in dog pulp after calcium hydroxide application. The teeth studied had narrow pulpal spaces. The cause of this persistence and the delay in the healing process may therefore have been due to circulatory disturbances and incisal degeneration in teeth with narrow pulps (24), the deep chemical cauterization effect of calcium hydroxide (0.2 to 0.5 mm), and the thinness of the pulp in certain teeth (4, 34). This finding is also consistent with previous studies which have found human or monkey tooth pulp to respond better than dog's pulp to calcium hydroxide application (3, 22).

In our calcium hydroxide specimens, the exposure area underwent a rapid vacuolar destruction, with no haemorrhage or fibrin organisation, leading to coronal pulp collapse. From the 30-day period onwards, the coronal pulp portion was emptied and separated from the subjacent living pulp by a well-developed dentine bridge along with a moderate chronic inflammation in the underlying pulp. Seltzer and Bender (30) believe that during pulp exposures in Class V cavity preparations, the bur could penetrate and dilacerate pulp tissue producing disturbances in the calcification process or even inducing pulp abscess. In that the Class V cavity in the present study, was located near the canal orifice. In the light of these and previous studies (35, 40), it would appear that the features of the cavity itself played a dictating role in determining the whole wound pattern that developed.

Attempts at bridge-like barrier formation, seen at some distance below the exposure site as early as 7 days, were abortive in specimens dressed with both Gelfix and Beriplast. Cox et al. (10) suggested that the wounded primate pulp may need more than an inert dressing to form a dentine bridge consistently. Indeed, Schröder (29) stated that a certain degree of stimulation is needed to induce deposition of mineral in the barrier.

Although Beriplast and Gelfix have been recognised as being biocompatible with soft tissues, there was necrotic evolution in these teeth at 7, 30, 60 and 90 days. There was severe inflammatory cell migration with large areas of necrosis in association with many abscess formations. This unfavourable response may not simply have been due to the shortcomings of these materials for pulp capping; it could also be due to circulatory disturbances and incisal degeneration, and/or thinness of the dog pulps used in this study. Even though special care was taken to avoid disturbances in the healing process, because of the translucency of Beriplast it was hard to curettage the dentinal walls to eliminate all traces of the material to facilitate the hermetic sealing of the cavity. Furthermore, Beriplast and Gelfix are non-setting materials, which makes them rather harder to apply than calcium hydroxide. Inadvertent pushing of Beriplast and Gelfix into the pulp space during placement or further cavity restoration flexing the floor of the cavity, possibly intensified mechanical injury to and compression of the pulp tissue and increased the possibility of a severe response.

Hermetic sealing of the pulp might also not have been achieved because of the physical properties of the test materials used. Dislodgment of the materials
or gaps occurring between the dressing materials and the walls of the cavities might have led to bacterial colonisation. This could have arisen from bacteria becoming entrapped in open dentinal tubules or the smear layer or to persistence of chronic inflammation with subsequent dramatic purulent evolution. It is therefore legitimate to assume that the induction of high cellularity adjacent to these materials, together with the resorbing dressing and tissue residues caused it to act like a deep caries entity, and a true pulp disease ensued. The preliminary data generated from this study should, however, be interpreted with caution because Gelfix and Beriplast are experimental materials and not manufactured especially as pulp dressings and, to the best of our knowledge, have not been assessed as pulp capping agents by others, which therefore prevents any comparison with previous data.

Under the conditions of this study, both Gelfix and Beriplast proved to be detrimental as pulp tissue as capping materials. This was in marked contrast to the calcium hydroxide under which the remaining living pulp tissue, seemingly aided by the anatomic configuration of the exposure site, detached earlier from the exposure site around the canal orifice with a well-formed dentine bridge, and maintaining a low-grade chronic evolution, showed a much more favourable response profile.

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ÖZET

Cerrahi işlemlerde kullanılan Gelfix ve Beriplast'ın, kualaj maddesi olarak köpek pulpasında kullanılanması

Cerrahi işlemlerde kullanılan iki ticari materyalın (Gelfix ve Beriplast), pulpa kualaj maddesi olarak kullanılma potansiyeli, histolojik olarak köpek dışlerinde incelemesini ve kalıший hidroksit ile karşılaştırılmasıdır. Beş adet köpeğin 60 adet gelişimini tamamlaması sürekli kestici disinde, S. sıfır kaviteler hazırlanmıştır. Standart pulpa acomo işlemi tamamlanmıştır sonra, kualaj materyalleri acık pulpa yüzeyi ile temas edecek biçimde yerleştirilmiştir. Daha sonra kaviteler, çınko oksit tıjenol simanı ve amalgam ile restore edilmiştir. 7, 30, 60 ve 90 günün sonunda, dışler çekilmişdir. Histolojik kesitler hazırlanmış ve ilılık, nekroz, dentin korupsu ve tamir dentini oluşumu açısından değerlendirilmiştir. İncelenen her iki madde, Gelfix ve Beriplast, şiddetti ilılıhi yapan oluştururken, kalıший hidroksitin oluşturduğu pulpa reaksiyonu geçici olarak iyi bulunmuş ve belirgin bir iyileşme kapasitesi sergilemiştir.

Table 1. Histologic evaluation score

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<th>MATERIAL</th>
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<th>INFLAMMATORY CELL INFECTION (×)</th>
<th>DEGENERATIVE CHANGES</th>
<th>BRIDGE-LIKE DENTIN FORMATION</th>
<th>TERTIARY DENTIN</th>
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<td>5(C)</td>
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<tr>
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<td>5(C)</td>
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<td>5(G)</td>
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<td>3</td>
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<td>1(C)</td>
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Fig. 1. Teeth treated with calcium hydroxide
A. 7 days: Pulp wound beneath the exposure undergoes liquefaction necrosis. The surrounding pulp shows moderate acute inflammation. (H.-E. x 10)
B. 30 days: Note complete collapse of the coronal pulp, remodelling of radicular pulp beneath the bridging dentine layer (big arrow), underlying moderate chronic inflammation, healing tertiary dentine deposition (small arrow), and atrophic pulp degeneration. (H.-E. x 10)

Fig. 2. Teeth treated with Gelfix
A. 7 days: Original wound, partly filled with debris, and surrounded by compressed necrotic tissue. Pulp horn is chronically inflamed. Note rootward extension of the inflammation under the zone in which a hard tissue layer is being formed (arrow). The remaining radicular pulp appears intact. (H.-E. x 10)
B. 7 days: Higher magnification of hard tissue forming zone. Underlying pulp is chronically inflamed. (H.-E. x 20)
C. 30 days: Under a slightly displaced dentine chip, large area of coagulation necrosis is seen. Note chronically inflamed underlying pulp and tertiary dentine formation (arrow). (H.-E. x10)

Fig. 3. Teeth treated with calcium hydroxide
A. 60 days: A dome shaped dentine bridge that mimics pulp horn has formed over the remaining pulp trunk near the centre is chronically inflamed pulp tissue, which is undergoing degeneration. (no nuclei are visible). Note tertiary dentine outline and underlying well-developed odontoblastic layer (arrow). (H.-E. x 20)
B. 90 days: Note pulp degeneration under the dentine bridge in association with chronic inflammation and heavy dentine deposition. (H.-E. x 20)
Fig. 4. Teeth treated with Gelfix.
A: 60 days: Pulp degeneration in association with severe chronic inflammation. No living odontogen layer is visible. (H.-E. x 10)
B: 90 days: Pulp degeneration in evolution. Note a cystic abscess cavity at the bottom (arrows). (H.-E. x 10)
C: 90 days: Higher magnification of apical portion. Note many abscesses forming throughout the radicular pulp (arrows). (H.-E. x 20)

REFERENCES

Fig. 5. Teeth treated with Beriplast after 60 (A) and 90 (B) day follow-up. Note total necrotic evolution associated with severe inflammation, internal resorption and abscess formation. (H.-E. x 10)


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