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HISTOLOGICAL EVALUATION OF PULP RESPONSE TO PULPINE NE VERSUS BIODENTINE AS DIRECT PULP CAPPING MATERIALS IN A DOG MODEL

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Introduction: The material used for pulp capping has a significant impact on the outcome of vital pulp therapy. This study compared the pulp tissue response to Pulpine NE versus Biodentine as direct pulp capping materials in a dog model.

Methods: Twenty-four teeth in two mongrel dogs (1-2-year-old) were used. In each dog (n=12 teeth), the dental pulps were exposed in 8 teeth (2 experimental groups, 4 teeth each) and left unexposed in 4 teeth (control group, n=4 teeth). A class V cavity was performed on the buccal surface of the selected teeth in the experimental groups. The exposed pulps were capped either with Pulpine-NE (group I) or Biodentine (group II). Then, the cavities were restored with Riva resin modified glass ionomer filling material. One dog was euthanized at 14 days after pulp capping and the second dog was euthanized after 45 days. Histological analysis of the continuity of dentin bridge, tissue disorganization and inflammatory reaction were statistically analyzed.

Results: The results revealed that Biodentine exhibited statistically significant higher dentin bridge formation than Pulpine NE after 14 and 45 days ($P<0.05$). Pulpine NE showed significant higher tissue disorganization than Biodentine after 45 days ($P=0.046$). The number of inflammatory cells was significantly higher in Pulpine NE samples than that of the Biodentine samples after 15 days ($P=0.042$).

Conclusions: Pulpine NE was capable of inducing reparative dentin when used as a direct pulp capping material. Nevertheless, Biodentine showed more efficient dentin bridge formation, tissue organization and anti-inflammatory potential than Pulpine NE.

Keywords: Dog, Biodentine, Dentine bridge, Pulpine-NE, Pulp capping.

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The authors declare no conflicts of interest.

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ÉVALUATION HISTOLOGIQUE DE LA RÉPONSE DE LA PULPE À LA PULPINE NE PAR RAPPORT À LA BIODENTINE COMME MATÉRIAUX DE COIFFAGE DIRECT DE LA PULP DANS UN MODÈLE DE CHIEN

Introduction: Le matériau utilisé pour le coiffage pulpaire a un impact significatif sur les résultats de la thérapie pulpaire vitale. Cette étude a comparé la réponse du tissu pulpaire à Pulpine NE par rapport à la Biodentine en tant que matériaux de coiffage pulpaire direct dans un modèle canin.

Méthodes: Vingt-quatre dents de deux chiens bâtards (âgés de 1 à 2 ans) ont été utilisées. Chez chaque chien (n = 12 dents), les pulpes dentaires ont été exposées sur 8 dents (2 groupes expérimentaux de 4 dents chacun) et laissées non exposées sur 4 dents (groupe témoin, n = 4 dents). Une cavité de classe V a été réalisée sur la surface buccale des dents sélectionnées dans les groupes expérimentaux. Les pulpes exposées ont été coiffées soit avec Pulpine-NE (groupe I), soit avec Biodentine (groupe II). Ensuite, les cavités ont été restaurées avec un matériau d'obturation en verre ionomère modifié à la résine Riva. Un chien a été euthanasié 14 jours après le coiffage pulpaire et le deuxième chien a été euthanasié après 45 jours. L'analyse histologique de la continuité du pont dentinaire, de la désorganisation tissulaire et de la réaction inflammatoire a été analysée statistiquement.

Résultats: Les résultats ont révélé que Biodentine présentait une formation de ponts dentinaires statistiquement significativement plus élevée que Pulpine NE après 14 et 45 jours ($P < 0,05$). Pulpine NE a montré une désorganisation tissulaire significativement plus élevée que Biodentine après 45 jours ($P = 0,046$). Le nombre de cellules inflammatoires était significativement plus élevé dans les échantillons Pulpine NE que dans les échantillons Biodentine après 15 jours ($P = 0,042$).

Conclusions: Pulpine NE était capable d'induire une dentine réparatrice lorsqu'elle était utilisée comme matériau de coiffage pulpaire direct. Néanmoins, Biodentine a montré une formation de ponts dentinaires, une organisation tissulaire et un potentiel anti-inflammatoire plus efficaces que Pulpine NE.

Mots clés: Chien, Biodentine, Pont dentinaire, Pulpine-NE, Coiffage pulpaire.

Introduction

When pulpal involvement occurs, direct pulp capping (DPC), pulpotomy, or even root canal treatment are regarded plausible therapeutic choices, depending on the exposure magnitude, time elapsed between exposure and management, and the surrounding environment [1]. Tricalcium silicate (TCS)-based materials have been developed to address the shortcomings of calcium-hydroxide (CH) [2-5]. TCS-based materials like Mineral Trioxide Aggregate (MTA) and Biodentine have been found to stimulate reparative dentin (RD) production at a faster rate and with higher structural quality than CH [6-8].

Biodentine is a modern biocompatible TCS-based substance that stimulates early mineralization by increasing Transforming growth factor-beta (TGF-ß1) release from pulpal cells [9]. It also enhances the synthesis of reparative dentin (RD) by increasing the odontogenic differentiation of dental pulp cells (DPCs) [10, 11]. Owing to its good handling characteristics, short setting time, biomineralization potential, better physical qualities, and reduced cost it could be an excellent DPC material [12].

There is a lot of interest these days in using natural materials to cure a variety of disorders. Propolis has been the subject of extensive inquiry during the previous decade. Pulpine NE is a novel biocompatible Propolis-based substance that could be a viable alternative to TCS-containing materials [13, 14]. Propolis is a commonly used medicine made up of plant resins, essential oils, beeswax, pollen, and organic compounds excreted by bees [15]. It possesses antioxidant, antibacterial, antifungal, immuno-regulatory and anti-inflammatory actions that may aid in the treatment and healing of exposed pulp [15, 16]. Dentin bridge (DB) has been observed in teeth capped by Propolis with mild or no pulpal inflammation [6, 16-18].

The most important component of Propolis is flavonoids, particularly Hesperidin. Flavonoids modulate the immunological response, reduce free radical production, and inhibit bacterial and fungal development, implying that this component possesses several beneficial biological effects [15]. Direct pulp capping with Hesperidin decreases pulp inflammation and promotes RD formation [19].

The material used for pulp capping has a significant impact on the outcome of vital pulp therapy. Therefore, inflammatory response, tissue organization and dentine bridge formation following pulp capping materials are the most commonly assessed criteria by histopathology [8, 14, 18].

There are scarce studies compared the pulp response of the currently available TCS materials to the naturally based ones such as Pulpine NE [13, 14]. Therefore, this study compared the histological reaction of healthy pulp to Pulpine NE when applied as DPC material to that of Biodentine. The null hypothesis was that the two capping materials (Pulpine NE and Biodentine) have no difference in pulp response when used for DPC in dogs' teeth.

Materials and Methods

Ethical approval

All international standards for the animals use and care were implemented. The experimental procedures were conducted after gaining the ethical approval from Research Ethics Committee, Faculty of Dental Medicine for Girls, Al-Azhar University, Egypt (REC17-01123).

Animal model

The sample size was determined based on earlier studies [20, 21] using the G*power software 3.1.9.2, where a large effect size of 1.38 was detected. The significance level (α-error) was set at 0.05 and the power (1- β error) was set at 0.8 using two-sided hypothesis test. The estimated sample size was 4 for each group at each evaluation period, summing up a total sample size of 24 teeth.

Two healthy male mongrel dogs were enrolled in this study. Their weight ranged between 18 and 20 kg and age ranged between one and two years. Both dogs had intact dentition. The dogs were randomly placed in separate kennels at the Veterinary Surgery Department, Faculty of Veterinary Medicine, Cairo University. They were identically placed in good ventilation, cleaning, feeding, and 12h light-dark cycle conditions. The dogs were fed on soft diet, soup, and bread twice daily.

Classification of the teeth

Twenty-four teeth in two dogs were used in this study. In each dog, three quadrants each containing four teeth (one incisor, one canine, and two premolars) were included, combining 12 teeth/dog. In each dog, the teeth (n=12) were divided into 2 experimental groups (I&II, 4 teeth each) according to the type of capping material used, where group I specimens were capped with Pulpine NE and group II specimens were capped with Biodentine. The control group represented intact teeth with no treatment to evaluate the histological features of their normal pulp (n=4). The dogs were randomly allocated to be euthanized at the assigned evaluation periods; subgroup A (after 14 days) and subgroup B (after 45 days).

Surgical procedures

Each dog was weighed to calculate accurately the required anesthetic dose. Following 12 hours of fasting; each dog was premedicated by subcutaneous injection of 0.05 mg/kg Atropine sulfate (Atropine sulphate 1%®, ADWIA, Egypt) and intramuscular injection of 1mg/kg Xylazine HCl (Xylaject 2%®, ADWIA, Egypt), 15-minutes before the procedure's initiation. At the time of operation, the dog was anesthetized intravenously with 5mg/kg Ketamine HCl (Ketamine®, EPICO, Egypt) injected into the cephalic vein via a cannula. Maintenance of general anesthesia was done by injecting an incremental dose of 2.5% Thiopentol sodium solution (Thiopental sodium®, EPICO, Egypt) intravenously as 25 mg/kg.

The whole procedures were performed under aseptic condition, where the teeth were disinfected with 0.2% Chlorohexidine mouthwash and cotton rolls were used for isolation. In the experimental groups, class V cavities were prepared on the facial surfaces, about 2 mm coronal to the gingival margin, using inverted cone bur # 2 (Mani, Inc, Japan) in a low-speed hand piece mounted on micromotor (Strong, SB-LS4C-022A, Korea) under copious sterile water spray. A standardized pulp exposure (approximately 0.8 mm in diameter) was performed by deepening the center of the pulpal floor using # 1 round carbide bur (Mani, Inc, Japan). Slight hemorrhage was observed and hemostasis was carried out by irrigation of the exposure site with sterile saline solution as well as a light pressure by sterile cotton pellets until the onset of physiological hemostasis (~ one min). After achieving hemostasis, the study groups' cavities were randomly assigned into 2 groups as follows:

Group I (Pulpine NE) in which the pulp exposures were capped with Pulpine NE that was mixed as instructed by the manufacturer. One spoon of powder was blended with three drops of liquid to form a homogenous consistency. The mix was applied directly over the exposed pulp using a sterile condenser.

Group II (Biodentine) in which the pulp exposures were capped with Biodentine, where five drops of liquid were utilized with one capsule of powder, then mixed in an amalgamator (3D Dental Optical Amalgamator, USA) at 4000-4200 rpm for 30 sec, as suggested by the manufacturer. Putty-like consistency was applied to the exposure site by an amalgam carrier and packed with a sterile condenser. The pulp capping materials used, manufacturer and their chemical composition are described in Table 1. Finally, the cavities were restored with Riva resin modified glass ionomer filling material (Riva Light Cure, HV, Australia) using led light curing device mini-s (Guilin Woodpecker Medical Instrument

Table 1: The pulp capping materials used, manufacturer, their chemical composition, presentation and mixing techniques.

Products	Manufacturer	Composition	Presentation	Mixing technique
Pulpine-NE (Eugenol-free, calcium-zinc based cement)	Hoffmann Dental Manufaktur GmbH Komtursraße, Berlin	**Powder**: Calcium compounds (Containing 1.9% CH) Zinc compounds. **Liquid**: Propolis. Ethanol.	Powder/liquid	Manual
Biodentine (Calcium silicate-based cement)	Septodont, Saint-Maur-desFossés Cedex, France	**Powder**: Tri- and Di- calcium silicate. Calcium carbonate. Zirconium-, Calcium- and Iron- oxides. **Liquid**: Water. Calcium chloride (Accelerator) Modified polycarboxylate (a water-soluble surfactant polymer).	Powder in a capsule/liquid	Mechanical

Co., Ltd., China) with an output 1000 mW/cm^2 for 20 sec.

Dogs were randomly labeled during the observation periods as 14-D and 45-D. All dogs were fed on a soft diet until the euthanasia time. For pain and infection control, both dogs were given intramuscular cefotaxime sodium at a dose of 10 mg kg and Diclofenac sodium at a dose of 1.1 mg kg once/day for 5 days after surgery [22].

Dogs were euthanized by injecting anesthetic overdose of 5% Thiopentol sodium solution (20ml) rapidly administrated via the cephalic vein. Thereafter, jaws were detached, and dogs' disposal was done through incineration in the medical incinerator at Faculty of Veterinary Medicine, Cairo University.

Histological evaluation

Jaws were fixed via 10% buffered formalin solution (Sigma, St. Louis, USA) for two-weeks after removal of the root apex to allow fixative penetration. Then, the specimens were demineralized for four-months in Morse's solution (American MasterTech Scientific, USA) that renewed every week [21].

The specimens were dehydrated by passing them through ascending concentrations of ethanol (from 70 to 100%), embedded in paraffin wax, then serially sectioned into sections with 5 μm thickness, via the capping material and pulp exposure site in facio-lingual plane. All sections were stained with hematoxylin-eosin (H&E) stain and evaluated by a blinded observer under a light microscope (Zeiss, Germany) attached to a digital camera (Leica, Germany) at magnification power (X40 and X200). Histological analysis of the continuity of dentin bridge, tissue disorganization and inflammatory reaction was performed. Photomicrographs were analyzed according to the criteria presented in Table 2, according to previous studies [23, 24].

Statistical analysis

Frequency of qualitative scores was expressed as numbers (N) and percentages (%). Data were statistically analyzed using version 20 of SPSS (SPSS Chicago, IL, USA). Chi square test was used for comparison between groups and within the same group at the assigned evaluation periods, where the significance level was set at $P < 0.05$.

Results

Control group

Histological examination of control intact teeth showed normal pulp architecture that consisted of loose connective tissue, collagen fibers and numerous blood vessels (BV) with no inflammatory cells. Intact well-arranged odontoblastic layer was observed overlying predentin layer. The demarcation line between primary dentin (PD) and RD was noticed (Figure 1a, b)

Subgroup A (After 14 days)

In group I (Pulpine NE), the histological analysis of the pulp revealed that no RD was seen at the exposure area. The underneath pulpal tissue lacked its normal architecture, where an intense inflammatory reaction was obvious. However, the odontoblastic layer was observed alongside the pulpal wall with their normal organization (Figure 2a, b).

In group II (Biodentine), the histological analysis of the pulp revealed partial/complete reparative DB formation at the exposure area that was partially lined with a layer of odontoblast-like cells. The pulpal tissue lacked the normal architecture and contained multiple enlarged BV engorged with RBCs as well as few inflammatory cells (Figure 2c, d).

Subgroup B (After 45 days)

In group I, the histological analysis of the pulp revealed that a thin layer of complete DB was obvious. Disturbance of the normal pulpal architecture was noticed which characterized by multiple BV with different sizes engorged with RBCs (Figure 3a, b).

In group II, a thick layer of complete DB with different degrees of stainability was formed. A well-arranged odontoblast-like cell layer was observed under the DB. The underneath pulpal tissue showed relatively normal architecture with a well-arranged odontoblastic layer and multiple pleomorphic dilated BV engorged with RBCs (Figure 3c, d).

Results of the statistical analysis

Data are presented in Figure 4 and tables 3-5.

Continuity of calcified barrier

For subgroups A and B, significant difference in DB formation scores was determined between the experimental groups, where group II exhibited significantly lower scores than group I, denoting partial/complete DB formation ($P=0.049$ and 0.046 for subgroups A and B, respectively).

Within group I (Pulpine NE), 0/4 specimens displayed complete DB formation after 14 days compared to 2/4 specimens after 45 days. While, 1/4 specimens exhibited complete DB formation after 14 days compared to 4/4 specimens after 45 days in group II (Biodentine). The differences were statistically significant ($P=0.049$ and 0.019 within groups I and II, respectively) as shown in table 3.

Tissue disorganization

Regarding subgroup A, no significant difference was determined in tissue disorganization scores between the two experimental groups ($P=1$). However, the difference between the experimental groups was statistically significant in subgroup B, indicating that the architecture of the pulpal tissue beneath the RD was relatively

Table 2: The histopathological evaluation criteria as described by Nowicka et al., and Mali and Mangala [23, 24].

Evaluation criteria	Scoring system	Definition
Continuity of calcified barrier	Score 1	Formation of complete dentin bridge.
	Score 2	Formation of partial DB (Extended > than 1/2 of the exposure site, but did not completely close it).
	Score 3	Formation of initial DB (Extended < than half of the exposure site).
	Score 4	No DB formation.
Tissue disorganization	Score 0	Presence of healthy tissues beneath the pulp capping material.
	Score 1	Evidence of odontoblasts or odontoblast-like cells, with disorganization of pulp tissue pattern or hyperactivity of odontoblasts, in the presence of central healthy pattern of the pulp tissues.
	Score 2	Presence of disorganization of all pulp tissue patterns.
	Score 3	Evidence of pulp necrosis.
Inflammatory reaction	Score 1	Absence or few of inflammatory cells (IC).
	Score 2	Mild inflammation (less than 10 cells).
	Score 3	Moderate inflammation (10-25 cells).
	Score 4	Severe inflammation (more than 25 cells).

Table 3: Frequency (N) and percentage (%) of the continuity of dentin bridge scores of the experimental groups within the evaluation periods.

Subgroups	Continuity of dentin bridge scores N (%)								*Statistical analysis*
	Group I (Pulpine NE)				Group II (Biodentine)				
Subgroup A (14 days)	Score 1	Score 2	Score 3	Score 4	Score 1	Score 2	Score 3	Score 4	$X^2 = 8$ P=0.049*
	0 (0%)	0 (0%)	0 (0%)	4 (100)%)	1 (25%)	2 (50%)	1 (25%)	0 (0%)	
Subgroup B (45 days)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	0 (0%)	X2 = 8.1 P=0.046*
Statistical analysis	$X^2 = 8$ P = 0.049*				$X^2 = 14.8$ P = 0.019*				

Significance level at P ≤ 0.05, *significant, ns=non-significant.

Table 4: Frequency (N) and percentage (%) of the tissue disorganization scores of the experimental groups within the evaluation periods

Subgroups	Tissue disorganization N (%)								*Statistical analysis*
	Group I (Pulpine NE)				Group II (Biodentine)				
Subgroup A (14 days)	**Score 0**	**Score 1**	**Score 2**	**Score 3**	**Score 0**	**Score 1**	**Score 2**	**Score 3**	$X^2 = 0$ P=1 ns
	0 (0%)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)	0 (0%)	
Subgroup B (45 days)	0 (0%)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	X2 = 8.1 P=0.046*
Statistical analysis	$X^2 = 0$ P=1 ns				X2 = 8.1 P=0.046*				

Significance level at P ≤ 0.05, *significant, ns=non-significant.

Table 5: Frequency (N) and percentage (%) of the inflammatory reaction scores of the experimental groups within the evaluation periods.

Subgroups	Inflammatory reaction N (%)								*Statistical analysis*
	Group I (Pulpine NE)				Group II (Biodentine)				
Subgroup A (14 days)	**Score 1**	**Score 2**	**Score 3**	**Score 4**	**Score 1**	**Score 2**	**Score 3**	**Score 4**	$X^2 = 8.3$ P= 0.042*
	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	
Subgroup B (45 days)	4 (100%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	0 (0%)	$X^2 = 0$ P=1 ns
Statistical analysis	$X^2 = 8.3$ P= 0.042*				$X^2 = 8.3$ P= 0.042*				

Significance at level P ≤ 0.05, *significant, ns=non-significant.

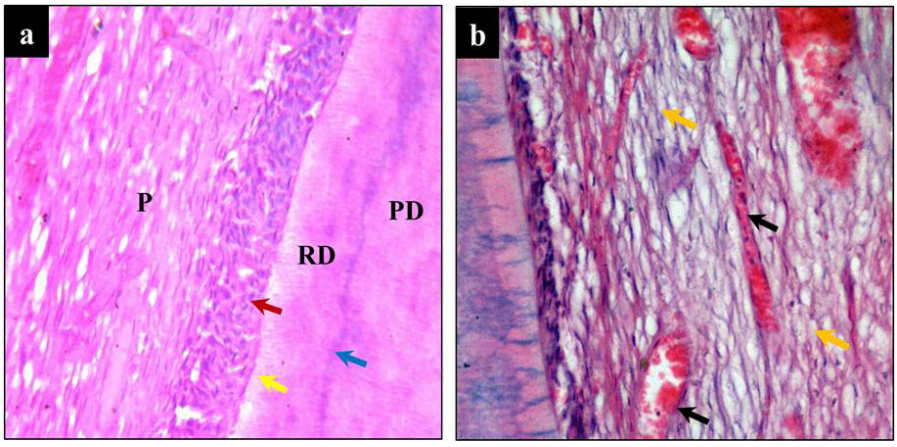

Figure 1: Photomicrographs of control intact teeth with normal pulp. (a) Showing the pulp core with well-organized odontoblastic layer (red arrow) that overlying predentin layer (yellow arrow) and the demarcation line (blue arrow) (H&E X40). (b) Showing the reticular collagen fibers (orange arrows) and blood vessels (black arrows) with no inflammatory cells (H&E X200). P: pulp; PD: primary dentin; RD: reparative dentin

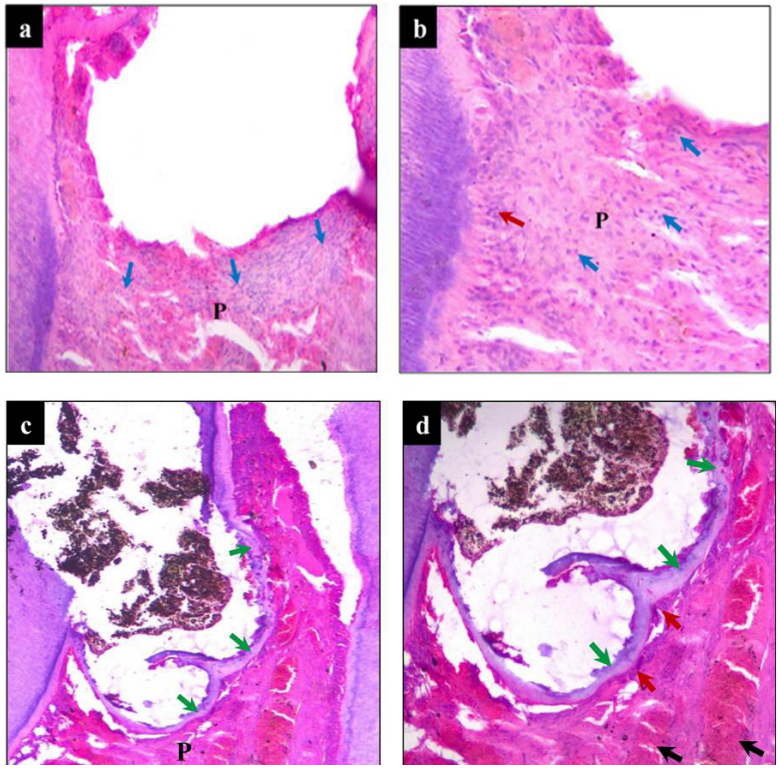

Figure 2: Photomicrographs after 14 days of pulp capping. (a, b) Group I (Pulpine NE) showing no dentin bridge formation, multiple inflammatory cells (blue arrows) and an organized odontoblastic layer (red arrow) along the pulpal wall (H&E, a: X40; b: X200). (c, d) Group II (Biodentine) showing odontoblast-like cell layer (red arrows) underneath the deposited dentin bridge (green arrows) and multiple dilated blood vessels (black arrows) (H&E, c: X40; d: X200). P: pulp

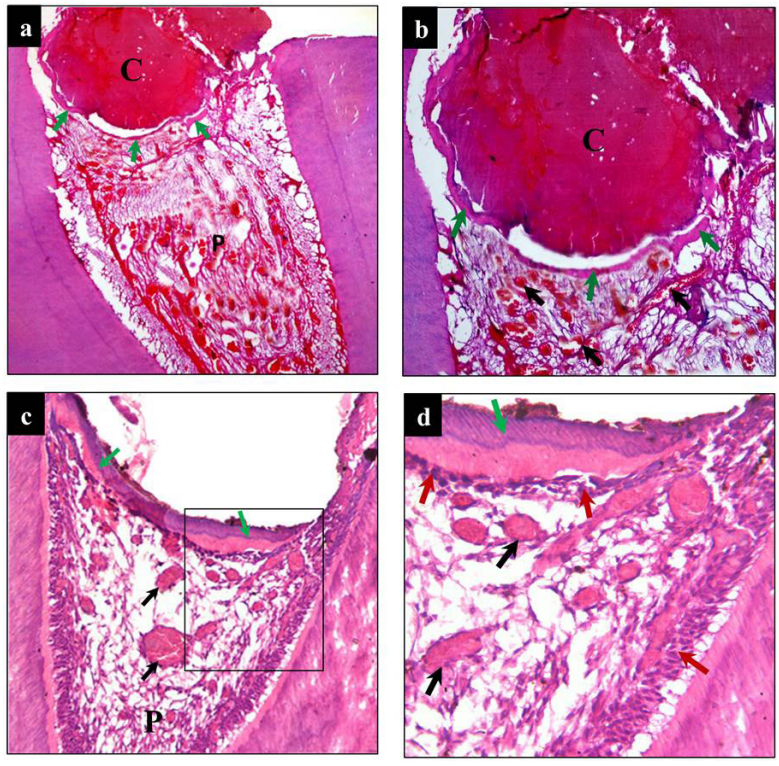

Figure 3: Photomicrographs after 45 days of pulp capping. (a, b) Group I (Pulpine NE) showing thin DB layer (green arrows), multiple blood vessels (black arrows) and disorganized pulpal architecture (H&E, a: X40; b: X200). (c, d) Group II (Biodentine) showing thick dentin bridge layer (green arrow), multiple pleomorphic blood vessels (black arrows), well-arranged odontoblast-like cell/odontoblastic layers (red arrows) and relatively normal pulpal architecture (H&E, c: X40; d: X200). C: capping material.

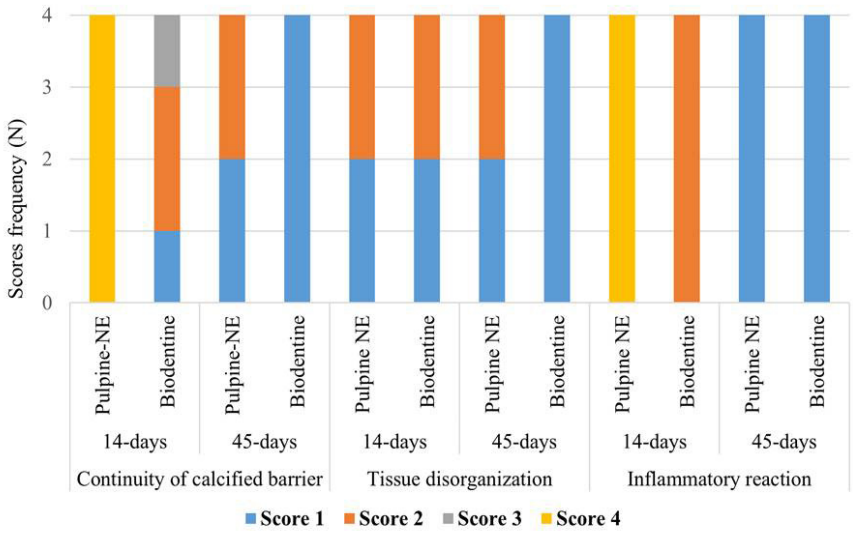

Figure 4: A bar chart comparing the frequency of continuity of calcified barrier, tissue disorganization and inflammatory reaction scores between the experimental groups at two evaluation periods (14 and 45 days).

normal in group II (P=0.046) as shown in table 4.

Inflammatory reaction

For subgroup A (after 15 day), the number of inflammatory cells was significantly higher in group I compared to group II (P=0.042). In subgroup B (after 45 days), the difference was statistically non-significant (P=1).

Within group I, all specimens showed severe inflammatory reaction after 14 days compared to minimal inflammation after 45 days. The difference was statistically significant (P=0.042). However, there was no significant difference between the two subgroups in group II (P=1) as shown in table 5.

Discussion

Direct pulp capping with CH has been the traditional treatment for many years; however, it is being phased out in favor of newer materials with more predictable clinical effects, such as calcium silicates [3, 10]. Natural materials produced from many sources have long been used in medicine, with several biological properties scientifically verified. Propolis is now being studied as a natural pulp capping material in dentistry [6, 17-19]. Therefore, the current study compared the histological reaction of pulp that was capped with a Propolis-based material (Pulpine NE) to that of Biodentine. The null hypothesis of this study was rejected because Pulpine NE and Biodentine capping materials had differences in their pulp responses.

This study utilized a dog model for phase validation of an innovative capping material (Pulpine NE) before being implemented in human clinical practice. The use of dogs is justified since their reparative dentinogenesis and healing processes are so comparable to those of humans [25]. Furthermore, dogs have a significant number of teeth per animal, enabling for the comparison of different capping materials in the same dog [8, 11, 14]. The 3rd incisor, canine, 2nd and 3rd premolars were chosen due to their good accessibility and adequate pulp size for histological evaluation [26].

When pulp exposure occurs as a result of caries, the pulp tissue is likely to be infected with bacteria during treatment, putting the reparative response at risk [1]. To avoid the involvement of confounding factors, this study was carried out under controlled experimental conditions using healthy teeth, as previously mentioned [5, 23].

The 14-day interval was chosen in this study based on earlier research findings showing hard tissue formation began after 2 weeks [6]. Furthermore, in keeping with prior studies, the current study assessed the influence of capping materials on pulp tissues over a 45-day period [9, 23, 27].

Biodentine caused significantly more DB formation than Pulpine NE in both subgroups A and B of the current investigation. After 15 days, the majority of specimens capped with Biodentine showed similar DB development [9, 28]. Biodentine has the advantage of enhancing DB formation in less time [11]. This favorable outcome could be attributable to a number of variables. The presence of calcium and silicon ions may induce dental pulp cells migration, proliferation, and differentiation into odontoblast-like cells via numerous molecular signaling pathways, including MAPK and Wnt/ß-catenin [9, 29]. Furthermore, Biodentine modulates TGF-ß1 that increases odontoblastic differentiation and mineralized nodule formation in pulp cells [9].

In addition, mechanical trituration of biodentine is advantageous. When biodentine powder and liquid are mixed with an amalgamator, the setting of the material is a hydration reaction. While Calcium silicates partially dissolve by adding the liquid, a hydrogel of hydrated silicate is produced. This will precipitate on the remaining Silicate particles' surface and in the spaces between the particles leading to a signi□cant decrease in the material's porosity and an increase in its compressive strength over time [30]. An earlier study concluded that both trituration and hand manipulation

techniques of biodentine exhibit microleakage, but the extent of microleakage was significantly less in biodentine manipulated mechanically when compared to hand manipulation [30]. This can be attributed to the fact that mechanical trituration produces a more homogenous mix than manual mixing. Furthermore, the water powder ratio will be altered in manual mixing resulting in nonhomogeneous mix [30].

Absence of DB in group I specimens after 15 days could be attributed to the need of Pulpine NE to be in direct contact with the pulp tissue for a longer period of time. This finding was in accordance with previous studies [14, 31]. In contrast, one study concluded that Propolis can induce DB formation after 14 days [17]. This may be due to the experimental model used in their study (rabbits) bears the least resemblance to canines and humans [25].

Although no DB formation was observed in the Pulpine NE group after 14 days, 50% of the specimens showed partial DB formation after 45 days, and the other 50% showed complete DB formation. As a result, the current study findings demonstrated that Pulpine NE had a good effect on DB formation with little pulpal inflammation after 45 days. These findings are consistent with those of a prior study [14]. This could be owing to the incorporation of two critical ingredients in Pulpine NE: CH, which is well known for its ability to repair the dentin-pulp complex as well as its antibacterial capabilities [1, 32]. Propolis, the second component, has been demonstrated to promote TGF-ß1, which may influence pulp tissue repair by stimulating fibroblast proliferation and increasing collagen deposition [33, 34]. Furthermore, Alkaline Phosphatase (ALP) is a key enzyme in mineral deposition. After 21 days of growth in ethanolic extract of Brazilian Propolis dental pulp cells, ALP expression increased [35]. Furthermore, Propolis extract suppresses apoptosis by limiting nuclear factor-kß (NFkß) translation into the nucleus, which prevents a severe fall in the number of fibroblasts in the pulp and inhibits the transcription of TNF-secreting genes [36].

Biodentine, when compared to Pulpine NE, promotes the production of thick RD lined with a well-arranged odontoblast-like cell layer in subgroup B. Other researches have found similar results [11, 21, 37].

The inflammatory response is the first stage of healing. When tissue is damaged, fibroblasts rush to the wound, proliferate, and make a huge amount of collagen matrix, which helps in the isolation and healing of the injured tissue [38]. Therefore, the initial inflammatory reaction of Pulpine NE after 15 days may reflect a favorable pulpal tissue response. This finding is in agreement with a recent previous [14].

The mild inflammatory response of Biodentine could be due to the reduction of pro-inflammatory cytokines release such as Interleukine 6 (IL-6) and Vascular Endothelial Growth Factor (VEGF) [39]. Moreover, Biodentine has been shown to lower Transient Receptor Potential Ankyrin 1 (TRPA1) functional activity, which plays a crucial role in the inflammation, and hence attenuate tumor necrosis factor TNF-α-induced TRPA1 expression [39]. Similar to our results, the mild inflammatory response of Biodentine was previously recorded [11, 21, 40].

Changes in outcomes between Biodentine and Pulpine NE could be attributed to changes in chemical composition, mechanical qualities, and sealing ability. Biodentine is TCS-based cement, whereas Pulpine NE is a calcium-based substance with a low concentration of CH. Because of its soluble nature, CH has a long-lasting irritating effect due to the constant release of calcium and hydroxyl ions. Conversely, Biodentine permits the release of a significant number of ions at the first setting, which limits ion release over time and offers more favorable conditions for pulp repair [10, 12]. The release of calcium ions from the DPC biomaterials stimulates the precipitation of calcium carbonate in the damaged area and thereby contributes to the initiation of mineralization. The pulp cells then begin to differentiate; these cells have odontoblast-like behavioral traits and start to produce a collagen-rich matrix that resembles predentin. Although one of the main substances released by DPC materials is calcium ions [10,41]. On the other hand, new data suggests that calcium ions serve essential roles in sustaining and regulating regular biological activities, as well as in the development of mineralized matrixes and the propagation of intracellular signaling pathways. Furthermore, calcium ions released by pulp-capping materials may have a role in the formation of reparative dentin, according to recent studies. These calcium ions are thought to be one of the main mediators of the mineralization process [42].

In addition, Biodentine has good sealing quality, which offers a predictable secondary barrier underneath the surface seal, reducing microleakage and pulpal irritation in addition to its superior mechanical qualities [10, 11].

Despite the fact that Pulpine NE induced a greater inflammatory response than Biodentine after 14 days, a substantial decrease in the frequency of IC was seen after 45 days. This is due to propolis, the major component of Pulpine NE, that has anti-inflammatory characteristics through reduction of the inflammatory response by blocking the lipoxygenase route of arachidonic acid metabolism

[15, 43]. Propolis also has the ability to induce TGF-ß1, which regulates the inflammatory response by acting as a regulator on immunocompetent cells such as lymphocytes, macrophages, and granulocytes [6, 17, 35].

Future studies on the effect of Pulpine NE on teeth with inflamed pulp should be conducted in order to generate a setting as near to reality as possible. Moreover, further studies are recommended to investigate the mode of action of Pulpine NE on the pulp tissue and to assess its immunohistochemistry reaction.

Conclusion

When employed as a direct pulp capping material, Pulpine NE was able to form reparative dentin, although Biodentine was more efficient in terms of dentin bridge building, tissue organization and anti-inflammatory capability.

Clinical relevence

Biodentine exhibits superior dentin bridge formation, tissue orgainzation and anti-inflammatory effect than Pulpine NE when used as direct pulp capping materials.

List of abbreviations

ALP: Alkaline Phosphatase, BV: Blood vessels; CH: Calcium hydroxide; DB: Dentin bridge; DPC: Direct pulp capping; DPCs: Dental pulp cells; H&E: Hematoxylin-eosin; IC: Inflammatory cells; IL6: Interleukine 6; MAPK: Mitogen-activated protein kinas; MTA: Mineral Trioxide Aggregate; NFkß: Nuclear factor-kß; PD: Primary dentin; RD: Reparative dentin; TCS: Tricalcium silicate; TGF-ß1: Transforming growth factor-beta; TNF: Tumor necrosis factor; TNF- α: Tumor necrosis factor alpha; TRPA1: Transient Receptor Potential Ankyrin 1; VEGF: Vascular Endothelial Growth Factor

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