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Assessment of biocompatibility properties of Calcium– Silicate Based pulp capping materials

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Abstract: Purpose: The objective of the study was to evaluate biocompatibility properties of calcium–silicate based pulp capping materials (MTA-Biodentine and Theracal). **Materials and Methods:** 27 males and females rabbits were selected to be used for the present study. The rabbits were classified into three mains groups. the roof of the pulp was removed and the pulptomy materials were mixed and the exposed pulps were capped with either of the three pulptomy agents (MTA, Biodentine or Theracal) "3 rabbits from each main group" at 1month, 2months and 3 months and the pulpotomized teeth evaluate the histopathological changes of the pulpal reactions to the selected materials under the light microscope using a definite scoring system. **Results:** The early response of pulp tissue to all tested materials was inflammatory reactions, of various degrees, that were subsided by time and Pulp tissue responses more favorably to both MTA and Biodentine compared to Theracal. **Conclusion:** The responses of pulp tissue to the tested materials are time dependant i.e. it get affected by increasing the duration of capping.
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1. Introduction

The maintenance of pulp vitality has been a challenge for restorative dentistry, yet it is essential for the preservation of the pulp–dentin complex. Vital pulp therapy aims to treat reversible pulpal injury and maintain pulp vitality and function. The injury may involve pulpal exposure and the application of a capping material can protect the pulp from additional injury, thereby facilitating healing and repair [1,2]. Calcium Hydroxide, is well known to be the most accepted material regarding the pulp capping materials. When calcium hydroxide is placed in contact with the pulp tissue, it preserves vitality, with no inflammatory response, stimulating the formation of a mineralized tissue barrier[3,4,5]. This coincides with the findings of Dominguez et al[6] who mentioned that different formulations of calcium hydroxide should be the materials of choice for pulp capping and pulpotomies, because of their antibacterial or odontoblast –like cells that form a hard tissue barrier in the pulp.

However, the effect of calcium hydroxide has been verified in numerous histological and clinical studies and each time a new material for capping or pulpotomy is suggested, the effect of the new material is compared with the effect of calcium hydroxide [7, 8]. Recently, materials as MTA and biodentine and theracal showed that it can stimulate dentinal bridging and appears to have particular promise as pulp-capping materials [7,9].

In view of the above considerations, the present study was conducted to evaluate the effect of MTA and Biodentine on the pulp tissue of rabbit' teeth and to compare it with Theracal.

2. Materials and Methods

27 males and females rabbits were selected. The animals were housed in veterinary surgery of the faculty of veterinary medicine, Cairo University. The animals had balanced diet and water. Under constant conditions of temperature and humidity, they were housed in 50cm^3 stainless steel wire lid covered polycarbonate cages that were placed in a room set up to provide simulated day and night cycles of 12 hour artificial lighting and 12 hour darkness. Animal experimentation was carried out in accordance with the guidelines set out by the Canadian Council on Animal Care and in coherence with the "Three Rs" of animal ethics.

The rabbits were classified into three mains groups: **Group I:** Pulpotomies were performed in (27) upper or lower incisors of 27 rabbits with MTA. **Group II:** Pulpotomies were performed in (27) upper

or lower incisors of 27 rabbits with biodentine. **Group III:** Pulpotomies were performed in (27) upper or lower incisors of 27 rabbits with Theracal.

The upper & lower permanent central incisor in each rabbit was used and all teeth were isolated with a rubber dam, and the operative field was disinfected with 2% chlorhexidine gluconate. On the facial surfaces, the access cavity was prepared coronal to the gingival margins with number 2 round carbide bur mounted on low speed hand piece under copious sterile water spray by gaining access to the pulp chamber, then the roof of the pulp was removed and the contents of the pulp chamber were removed by means of large spoon excavator until the entrance of the root canals were identified. Bleeding was controlled by cotton pellet moistened with sterile saline until the physiologic hemostasis occurred. The pulptomy materials were mixed according the manufacturer instructions and the exposed pulps were capped with either of the three pulptomy agents (MTA, Biodentine or Theracal) according to its group. Each group was subdivided into three subgroups: A, B and C according to time of sacrification for evaluation "3 rabbits from each main group" at 1month,2months and 3 months: The pulpotomized teeth will be extracted for preparation of histologic sections The specimens were put immediately in a fixative; the most common one used was 10% formalin for 48-72 hours. After adequate fixation in 10% formalin solution, the specimens were washed under running tap water overnight to remove the excess of the fixative. The specimens was immersed in 10% EDTA for 4-5 weeks, then they were washed again under running tap water and Water was removed from the tissue gradually by putting it in ascending grades of alcohol (i.e, 50%, 70% and 90%) then in absolute alcohol. Since paraffin and alcohol are not miscible, the tissue was put in two changes of xylene (clearing agent) which is miscible with both alcohol and paraffin. It also made the tissue translucent. When xylen was completely replaced the alcohol in the tissue, and the specimens became clear, they were ready to be infiltrated with paraffin. They was removed from the xylene and placed in a dish of melted, embedding paraffin, and the dish was put into a constant temperature oven, regulated to about 60^0c for 2-3 hours. As the specimens were completely infiltrated, They were removed from the dishes of melted paraffin with a warm forceps and were placed in the center of a box of melted hard paraffin, the bottom of which was the surface of cutting. By the use of microtome, The paraffin embedded specimens were serially cut in a buccolingual plane parallel to the tooth vertical axis through the cavity preparation and the pulp into sections of 5 microns thickness showing the deepest part of the cavity and the underlying pulp. A short length of paraffin ribbon was floated in a pan of warm water (about 20^0c). The prepared slide was slipped under the ribbon and then lifted from the water with the ribbon, which contained the tissue sections arranged on its upper surface. The slide was placed on a constant temperature drying table which was regulated to about 37-42 ˚C, so that the sections adhered to the slide. The slide was then allowed to dry on this table. The staining technique: using Haematoxylin and eosin staining (Luna, H L. 1968) to evaluate the histopathological changes of the pulpal reactions to the selected materials under the light microscope using a definite scoring system.

3. Results

Histologic evaluation

The microscopic examination of the pulpotomized teeth of the rabbits of studied groups (MTA, Biodentine and Theracal revealed the following results:

In the first month:

Group I: (Biodentine group)

The initial reaction of the pulp to the material in 45% of specimens (4 specimens) was a thin layer of partially calcified dentin matrix (grade 1 hard tissue formation) that accompanied by a weak inflammatory reaction (grade o inflammatory cell response) limited to the pulpotomy site characterized by the presence of congested and dilated blood vessels and few mononuclear cells.

Group II: (MTA group)

Two of specimens (22%) showed destruction in the odontoblastic cell layer in the middle portion of the pulp and no dentin bridge formation (grade 0 hard tissue formation). Three specimens (33%) showed mild inflammation (grade 1 inflammatory cell response) involved the coronal and middle portions of the radicular pulp characterized by the presence of congested and dilated blood vessels and the infiltration of acute inflammatory cells as neutrophils in addition to some areas of necrosis below the exposed area, while three of specimens (33%) showed moderate inflammation (grade 2,3 inflammatory cell response) limited to the coronal portion of the pulp in their pulps.

Group III (Theracal):

The initial reaction of the pulp to the material in 45% of specimens (4 specimens) was a necrotic zone just beneath the material. This zone was followed by a thin layer of partially calcified dentin matrix (grade 1 hard tissue formation) that accompanied by a slight moderate inflammatory reaction (grade 1 inflammatory cell response) limited to the pulpotomy site characterized by the presence of congested and dilated blood vessels and few mononuclear cells. 55% of specimens (5 specimens) showed absence of the dentin bridge (grade 0 hard tissue formation) but with

presence of a necrotic zone below the exposed area and a moderate inflammatory reaction (grade 2 inflammatory cell response) mediated by few mononuclear cells.

After one month Dentinal bridge formation it was confirmed that there was statistically significant difference between the responses of teeth to all material groups (chi square test p<.05)

After one month Inflammatory response reaction showed that it was confirmed that there was statistically significant difference between the responses of teeth to all material groups

(chi square test p<.05)

In the second month:

Group I: (Biodentin group)

Four specimens (55%) showed the formation of completely formed dentin bridge (grade 2 hard tissue formation). four specimens of them exhibited a slight to a moderate inflammation (grade 1 inflammatory cell response) below the pulpotomy site but limited to the coronal portion of the radicular pulp with the presence of polymorphonuclear cells and machrophages.

Group II: (MTA group)

One specimen showed areas of necrosis in the coronal and middle two thirds of the pulp and extensive inflammatory reaction (grade3 inflammatory cell response) affecting most of the pulp characterized by the infiltration of chronic inflammatory cells, mainly plasma cells and lymphocytes, congested and dilated blood vessels in addition to some areas of necrosis below the exposed area, while seven specimen (77%) showed moderate inflammatory reaction extended to the middle one third of the pulp (grade 2 inflammatory cell response).

Six specimens (66%) showed the formation of completely formed dentin bridge (grade 2 hard tissue formation).

Group III (Theracal):

Three of specimens (40%) showed destruction in the odontoblastic cell layer in the middle portion of the pulp.

The reaction of the pulp to the material in 55% of specimens (6 specimens) was thin layer of partially calcified dentin matrix (grade 1 hard tissue formation) that accompanied by a weak inflammatory reaction (grade 0 inflammatory cell response) limited to the pulpotomy site characterized by the presence of congested and dilated blood vessels and few mononuclear cells.

After 2 month Dentinal bridge formation it was indicted that there was statistically significant difference between the responses of teeth to all material groups (chi square test p<.05)

After 2 month Inflammatory response reaction showed that it was indicted that there was statistically significant difference between the responses of teeth to all material groups (chi square test p<.05)

In the third month:

Group I: (Biodentin group)

Five specimens (55%) showed completely formed dentin bridge (grade 3 hard tissue formation) with a slightly inflammatory reaction extended in the coronal and the middle portions of the radicular pulp (grade 1 inflammatory cell response) characterized by the infiltration of plasma cells, lymphocytes and macrophages in two specimens (fig. 1).

*pulps showed odontoblastic cell layer with normal integrity (fig. 1).

* There is no any evidence of the presence of internal resorption or pus cells.

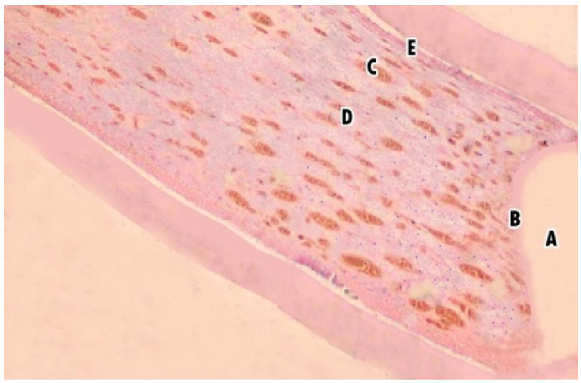

Figure (1): Group I; Photomicrograph of the tooth and its components from the inner, Biodentin material space **(A)**, calcific bridge **(B)**, dilated blood vessel **(C)** pulp tissue **(D)** and odontoblastic cell layer with normal integrity **(E)**. **(H & E Stain X 100).**

Group II (MTA)

Histopathological evaluation (for evaluation of the inflammatory cell response & dentin bridge formation):

Four specimens (45%) showed the formation of completely formed dentin bridge (grade 2 hard tissue formation). four specimens of them exhibited a slight to a moderate inflammation (grade 1 inflammatory cell response) below the pulpotomy site but limited to the coronal portion of the radicular pulp with the presence of polymorpho nuclear cells and machrophages, while the other specimen revealed a moderate inflammatory reaction extended to the middle one third of the pulp (grade 2 inflammatory cell response) with dilatation of blood vessels.

Group III (Theracal):

Four specimens (45%) showed the formation of completely formed dentin bridge (grade2 hard tissue formation). four specimens showed a slight to a moderate inflammation (grade 1 inflammatory cell response) below the pulpotomy site but limited to the coronal portion of the radicular pulp with the presence

of polymorpho nuclear cells and machrophages, while the other specimen revealed a moderate inflammatory reaction extended to the middle one third of the pulp (grade 2 inflammatory cell response) with dilatation of blood vessels.

All pulps characterized by presence of slight inflammation.

After 3 month Dentinal bridge formation it was proven that there was statistically significant difference between the responses of teeth to all material groups (chi square test p<.05)

After 3 month Inflammatory response reaction showed that it was proven that there was statistically significant difference between the responses of teeth to all material groups (chi square test p<.05)

Table (1): Frequent distribution (%) of inflammatory response scores for different groups as function of evaluation time.

Variable		1st month			2nd month			3rd month		
		Biodentine	MTA	Theracal	Biodentine	MTA	Theracal	Biodentine	MTA	Theracal
Inflammatory response	Sample 1	0	3	4	0	2	4	0	2	0
	Sample 2	0	1	0	0	2	0	0	1	0
	Sample 3	1	1	1	1	2	0	0	1	0
	Sample 4	1	3	2	1	3	1	1	2	2
	Sample 5	0	3	2	1	2	1	2	1	2
	Sample 6	0	4	2	0	3	1	0	2	0
	Sample 7	2	1	0	1	2	0	2	2	2
	Sample 8	0	2	4	0	2	2	0	2	2
	Sample 9	0	0	3	0	2	2	0	0	1
Frequent distribution of scores %	Score 0	66.67	11.11	22.22	55.56	0	33.33	66.67	11.11	44.44
	Score 1	22.22	33.33	11.11	44.44	0	33.33	11.11	33.33	11.11
	Score 2	11.11	11.11	33.33	0	77.78	22.22	22.22	55.56	44.44
	Score 3	0	22.22	11.11	0	22.22	0	0	0	0
	Score 4	0	11.11	22.22	0	0	11.11	0	0	0

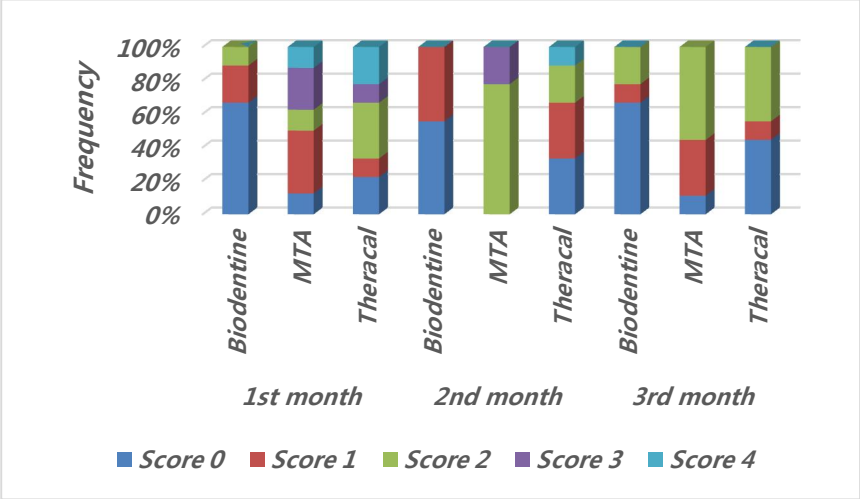

Figure (2): Stacked column chart of inflammatory response scores % for different groups as function of evaluation time

Effect of material; it was verified that there was statistically significant difference between the responses of teeth to all material groups (chi square test p<.05)

Effect of evaluation time; it was verified that there was statistically significant difference between the responses of teeth to evaluation time subgroups (chi square test p<.05).

4. Discussion

Vital pulp procedure, namely; pulp capping is performed frequently by dentists, most likely on weekly basis. Predominantly, this procedure is taught in dental school as temporary treatment on curiously and mechanically exposed teeth. However, some authors have suggested that vital pulp therapy treatments can be permanent. Because the pulp has enough vital tissue[1], advocated that pulp-capping

procedures could be performed successfully on an asymptomatic carious exposure. **(Haskel et al. 1978)**[2] supported this concept that proved that asymptomatic carious exposures could survive an average of 12 years after pulp capping. The development of newer materials that are biocompatible, bactericidal inductive of a reparative process, and have better sealing properties could render these treatments long term.

MTA is used to seal off exposed pulps and various communications between the root canal system and surrounding tissues for a variety of indications such as root-end filling, perforation repair, and apexification[10]. And Its ability to stimulate formation of a dentin bridge, consequently leading to pulp healing has been well demonstrated in previous studies [11]. MTA has excellent biocompatibility without mutagenic potential, sealing capacity and the formation of mineralized tissue.[12]

However, MTA has several disadvantages as a pulp-capping material because it has a long setting time and tendency to become discolored[13] and needs to be protected with other materials.

Biodentine, new calcium-silicate-based cement has been developed to improve some MTA drawbacks the advantage of Biodentine over MTA is handling, high viscosity, shorter setting time (12 minutes), and better physical properties [14], This material stimulates the deposition of hydroxyapatite on its surface when exposed to tissue fluids [15], presents color stability [13], is not genotoxic [16], and has low cytotoxicity, preserving gingival fibroblast viability [17]. In the few in vitro studies available so far, Biodentine presented compatibility to dental pulp cells and stimulated the formation of tertiary dentin [18,19,20]. It induced the differentiation of cultured pulp cells into odontoblast-like cells[19] and mineralized foci formation.

Theracal was developed in attempt to overcome the disadvantages of MTA as a pulp capping material as a calcium silicate-based, light-curable material.

It is a resin modified calcium silicate-based material that has exhibited calcium release properties and alkalinity[21]. Although TheraCal has low solubility as a pulp capping material[22], its interaction with the underlying pulp cells has not been reported in depth. The interaction between pulp capping materials and the exposed pulp tissue is important during the initiation and development of pulpal healing and reparative dentine formation.

Histopathological evaluation of TheraCal treatment in first month showed that The initial reaction of the pulp to the material in 45% of specimens (4 specimens) was a necrotic zone just beneath the material. These findings might be attributed to the presence of resin in its composition. It has been reported that up to 50% of methacrylate monomer double bonds remain unreacted in resin polymers[23,24]. The non-polymerized monomers in the composite represent a significant risk when they leach out from the composite and reach dental pulp[25,24]. This indicates that it is impossible to obtain complete polymerization, particularly if the material is applied in a humid environment as in direct pulp capping. Similarly, resin components of Thera Cal including HEMA, BisGMA, TEGDMA, and UDMA may remain non-polymerized after contact with pulp tissue, and these are known to be cytotoxic for pulp fibroblasts [26]. Thus, incomplete dentinal bridge formation may be due to monomers leaching from the material. Indeed, it has been demonstrated that nontoxic concentrations of monomers inhibit the secretion of dentin sialoproteins and osteonectin and their accumulation in endoplasmic reticulum [27]. Because these proteins are involved in the mineralization process, inhibition of their secretion may explain decreased mineralization, while histopathological evaluation of MTA treatment in first month showed that some of specimens showed moderate inflammation involved the coronal and middle portions of the radicular pulp characterized by the presence of congested and dilated blood vessels and the infiltration of acute inflammatory cells as neutrophils in addition to some areas of necrosis below the exposed area, while other specimens showed slight to moderate inflammation limited to the coronal portion of the pulp in their pulps and a thin layer of partially calcified dentin matrix. These findings might be attributed to high alkalinity of MTA, whose pH is 12.5 after 3 h[28,29] i.e. after being mixed with water, MTA generates, in its unset stage, a rather high pH, which causes cell coagulation, and longer setting time, therefore, provides ion release for a longer time. Moreover, hydration of the calcium oxide contained in MTA may result in an exothermic reaction[15,30], which could produce less favorable conditions for pulp repair.

Regarding Histopathological evaluation of Biodentine treatment in first month might be attributed to presence of tricalcium silicate which is one of the main components of Biodentine that cause stimulation of cell proliferation and differentiation[14], its sealing ability and it is ability to release of transforming growth factor b1this factor is involved in recruitment of pulp stem cells and their odontoblastic differentiation [31,20,24].

AT the 3 months period, MTA group showed in 60 % of samples more dentin bridge formation was noticed due to antibacterial property, and excellent sealing ability, It stimulates the production of cytokines in human osteoblasts, allows good adherence of the cells to the material, thereby playing an active role in dentin-bridge formation.[32,33].

Regarding Theracal it was proved that it has more calcium than MTA[21,34] These calcium ions play an important role in formation and mineralization of hard tissues in 50 % of samples[34].

While Biodentine group 80 % of samples showed formation of completely formed dentin bridge with a slightly inflammatory reaction and all pulps showed odontoblastic cell layer with normal integrity because of excellent sealing capacity of Biodentine [30,32,35] and high degree of biocompatibility[22].

We can conclude that the success rate of capping materials (Biodentine, Theracal and MTA) depend on several factors such as host, the capping material, and the operator, including the initial condition of the pulp tissue, the absence of microbial contamination, the nature of the materials, and the rigorous execution of the technical steps.

From the result of the present study, the following conclusions can be drawn:

1. All tested materials have the ability to enhance pulp tissue repair by induction of reparative dentinal bridge at the exposure site.
2. The early response of pulp tissue to all tested materials was inflammatory reactions, of various degrees, that were subsided by time.
3. Pulp tissue responses more favorably to both MTA and Biodentine compared to Theracal.
4. The responses of pulp tissue to the tested materials are time dependant i.e. it get affected by increasing the duration of capping.

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