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Influence Of MTA And Biodentin On Pulp Tissue As Direct Covering Agents – A Histopathological Analysis

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Abstract
Caries represents a destructive process that leads to progressive demineralization of the inorganic part of the tooth, followed by enzymatic disintegration of the organic component of dental tissues. Having in mind that carious activity can be stopped if we stop the demineralization process, it is clear that the objectives of carious treatment are directed toward eliminating the etiological factors and stimulating the regeneration of dental tissues Considering that preserving the vitality of pulp tissue is beneficial for the patient and challenging for the dentist, the therapist's attention is directed toward finding a material that will be biocompatible, possess an antibacterial effect, and also stimulate the formation of reparative dentin. Based on this knowledge, the aim of this paper is to histopathologically monitor the response of healthy human dental pulp after the application of Biodentine directly onto the exposed pulp and to compare this response with the pulp's response to capping with MTA, applied directly to the pulp exposure.
For the investigations we used the following materials: MTA and Biodentine, as a means of direct covering of pulpal exposure. The teeth selected for conducting the histopathological analyses were vital, and the indication for extraction was for orthodontic reasons. We obtained the patient's consent for carrying out these analyses.
After performing the histopathological analyses, the dental samples were examined using a light microscope at various magnifications. The presence of an inflammatory cellular response was observed, and it was assessed whether there was the formation of solid dentin tissue, the presence of hyperemia, or necrotic changes accompanied by disintegration of the pulp tissue.
After analyzing the obtained data, we made a comparison between the results obtained from different materials for direct pulp capping and formed the final conclusions, which allowed us to adopt a positive stance on the justification for their applied use.
Keywords: *Caries, Biodentine, MTA, histopathological analyses, direct pulp covering*

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I. Introduction

The goals of caries treatment are focused on eliminating the etiological factors and stimulating the regeneration of dental tissues. In recent years, significant progress has been made in the field of caries disease treatment, focusing on the remineralization of early carious lesions, as well as the biological behavior of the pulp-dentin complex after the application of certain medications.

Direct pulp exposure with reversible pulp inflammation, which may occur during cavity preparation, trauma, or caries excavation, can be a challenge but also a problem if an accurate diagnosis is not made before the therapy process begins.

Direct pulp capping can be defined as the covering of an exposed but clinically normal pulp with the absence of signs and symptoms indicative of pulpitis. This procedure is non-invasive and simple, with the sole aim of maintaining vital pulp tissue. (1) One of the main roles of pulp tissue is the physiological secretion of dentin throughout life, as well as its ability to regenerate dentinogenesis to protect itself in the event of external injuries.

Many researchers have been investigating the process of pulp tissue healing, and recent advances in biotechnology are opening new possibilities for the development and production of materials that, when applied to pulp tissue, promote reactive and reparative dentinogenesis, as well as the revascularization of infected canals. The success of the therapy depends on the extent to which the pulp cell population can survive and the ability of those cells to adequately respond to the carious insult and initiate the appropriate reparative process. (2) In case of pulp tissue injury, the subsequent reparative response is the deposition of a tertiary dentin matrix, either reactive or reparative dentin. The direction of dentin secretion depends on the intensity of the initial response and the conditions under which the newly formed dentin matrix is deposited. Generally, reactive dentin is produced by pre-existing odontoblasts, while reparative dentin is secreted by newly differentiated odontoblastic cells. (3) Therefore, reparative dentinogenesis is a much more complex process than reactive dentinogenesis and is a

subsequent process after cavity preparation, where pulp tissue becomes exposed. In such situations, the clinician must decide whether to apply a direct capping agent or proceed with pulpectomy. ()

The concept of vital pulp therapy is based on the use of biological principles to maintain the vitality of the pulp. Therefore, it is necessary to understand the pathophysiological processes occurring in the pulp-dentin complex, but also to consider the key factors contributing to the success of the therapy. These factors include the patient's age, their general health condition, the absence of previous painful symptoms, a small area of exposed pulp, minor pulp hemorrhage, and a good pulp response to external stimuli. (1, 5, 6).

Currently, research is focused on finding a bioactive material that will continuously stimulate cellular reparative mechanisms to form a biologically stable, reparative dentin bridge. Clinicians use many materials and techniques for direct pulp capping, including Ca(OH)2, hydrophilic resins, resin-modified glass ionomer cements, tricalcium phosphates, and more recently, mineral trioxide aggregate (MTA) and the bioactive silicate cement Biodentine.

Objective

Considering that preserving the vitality of pulp tissue is beneficial for the patient and challenging for the dentist, the therapist's attention is directed toward making the correct indication and decision on whether to perform direct capping of the exposed pulp tissue. Therefore, the need arises to find a material that will be biocompatible, possess an antibacterial effect, and also stimulate the formation of reparative dentin. Based on this knowledge, the aim of this paper is to histopathologically monitor the response of healthy human dental pulp after the application of Biodentine directly onto the exposed pulp and to compare this response with the pulp's response to capping with MTA, applied directly to the pulp exposure.

II. Materials And Methods

For the investigations, we used the following materials: Calxyl, MTA, and Biodentine. The type of material examined, its brand name, manufacturer, and chemical composition are presented in Table 1.

Material	Manufacturer	Chemical Composition
MTA	Dentsply, Tulsa Dental Products, Tulsa, OK, USA	Portland cement, bismuth oxide
Biodentine	Septodont, Saint Maur des Fosses, France	Powder: Tricalcium silicate, Calcium carbonate, Zirconium dioxide; Liquid: Calcium chloride, water reducer, water

Table 1. Materials Included in the Study

Picture 1. MTA Dentsply; Biodentine Septodont

Histopathological Analysis

	a. First group ***MTA***			*б Second group* ***Biodentine***	
region	**I after 8 days**	**II after 30 days**		**III after 8 days**	**IV after 30 days**
premolars	1	3		2	4
molars	5	3		4	2
tottal	**6**	**6**		**6**	**6**

Table 2. Distribution of Samples Included in the Histopathological Examination, where a Direct Pulp Capping Agent was Applied via Pulp Exposure: a. MTA b. Biodentine

The histopathological analysis was conducted at the Institute of Pathohistology in Skopje. The teeth included in this study were indicated for extraction for orthodontic reasons. When selecting the teeth from the patients, the following criteria were applied:

- The age of the patients ranged from 18 to 30 years.

- The patients were free from systemic diseases.
- The teeth had clinically normal pulp, without the presence of caries or any restorations.
- There were no periodontal changes in the teeth.

A total of 24 teeth were included in these analyses, and depending on the material used for direct capping as well as the duration of application, they were divided into four groups:

1. Teeth with MTA as the direct capping material, extracted 8 days after the application of the material.
2. Teeth with MTA as the direct capping material, extracted 30 days after the application of the material.
3. Teeth with Biodentine as the direct capping material, extracted 8 days after the application of the material.
4. Teeth with Biodentine as the direct capping material, extracted 30 days after the application of the material.

Two intact teeth were selected as a negative control group, where no pulp exposure or direct capping was performed.

The patients had previously signed consent forms after being explained the purpose for which the extracted teeth would be used.

Before starting the procedure, local anesthesia was applied to all patients, followed by the preparation of Class I cavities. Afterward, a sterile steel bur was used to perform trepanation. Once the hemorrhage was controlled with sterile cotton, the direct capping material was applied to the pulp exposure in a layer no thicker than 1.5 mm (MTA in groups 1 and 2, and Biodentine in groups 3 and 4). The cavities were then immediately sealed with Chemfil – glass ionomer (Dentsply) and the composite material SYNERGY®D6 (COLTENE®).

After 8 days, the teeth from groups 1 and 3 were extracted, and after 30 days, the teeth from groups 2 and 4 were extracted. The teeth were then longitudinally sectioned and immersed in a solution that performs rapid demineralization of the dentin (Osteomol), where they remained for 3-5 days, depending on whether they were ready for tissue sectioning with a thickness of 5 µm and staining using the Brown-Bren or Hematoxylin-Eosin method. They were then analyzed with a light microscope under different magnifications.

The pulp response was followed according to the following criteria:

- Observation of the presence of an inflammatory cellular response.
- Analysis of the morphology and thickness of the dentin bridge.
- Evaluation of whether solid dentin tissue formation occurred.
- The presence of hyperemia was determined based on the number of blood vessels.
- Monitoring for necrotic changes (abnormal cells, disintegration of pulp tissue, protein denaturation).

Characteristics	Category	Grading		
		I (poor)	II (medium)	III (best)
Dentin Bridge	Thickness	<0.1 мм	0,1-0,25 мм	>0,25 мм
Pulp inflammation	Type	Acute and chronic inflammation	Chronic inflammation	No inflammation
Other Histological Characteristics	Calcification	Diffuse calcification	Denticles	None

Table 3. Grading of Histological Characteristics after Application of Direct Capping Agents

III. Results

In this section of the research, the results of the histopathological analyses performed on 12 samples where direct capping was done with MTA and 12 samples where Biodentine was used as a direct capping agent are presented. For tracking and analyzing various characteristics of the histological preparations in each of the samples, the results are shown in separate tables and charts for the MTA group samples, while separate tables and charts present the data obtained from the analysis of teeth capped with Biodentine.

Table 4 and **Charts 1, 2, 3, and 4** present the distribution of samples where direct capping was performed with MTA. In group 1, the teeth are shown where MTA acted as the direct capping material 8 days prior to tooth extraction, while in group 2, the direct capping material remained for 30 days before the tooth was extracted.

		MTA					
		Group 1 (after 8 days)			Group 2 (after 8 days)		
Characteristics	Category	I	II	III	I	II	III
Dentin Bridge	Thickness	3	3	0	0	2	4
Pulp inflamation	*Type*	0	2	4	0	1	5

Other Histological Characteristics	***Calcification***	**2**	**0**	**4**	**2**	**0**	**4**

Table 4. Distribution of Samples Included in Histopathological Examinations Where MTA was Applied as a Direct Capping Agent via Pulp Exposure

In terms of dentin bridge thickness, in 3 of the samples from the first group, the thickness of the dentin bridge is less than 0.1 mm (I), while in the remaining 3 from the same group, its thickness ranges between 0.1-0.25 mm (II). In the second group, 2 of the teeth have a dentin bridge thickness ranging between 0.1-0.25 mm (II), while in the remaining 4, the thickness exceeds 0.25 mm (III). Chart 1

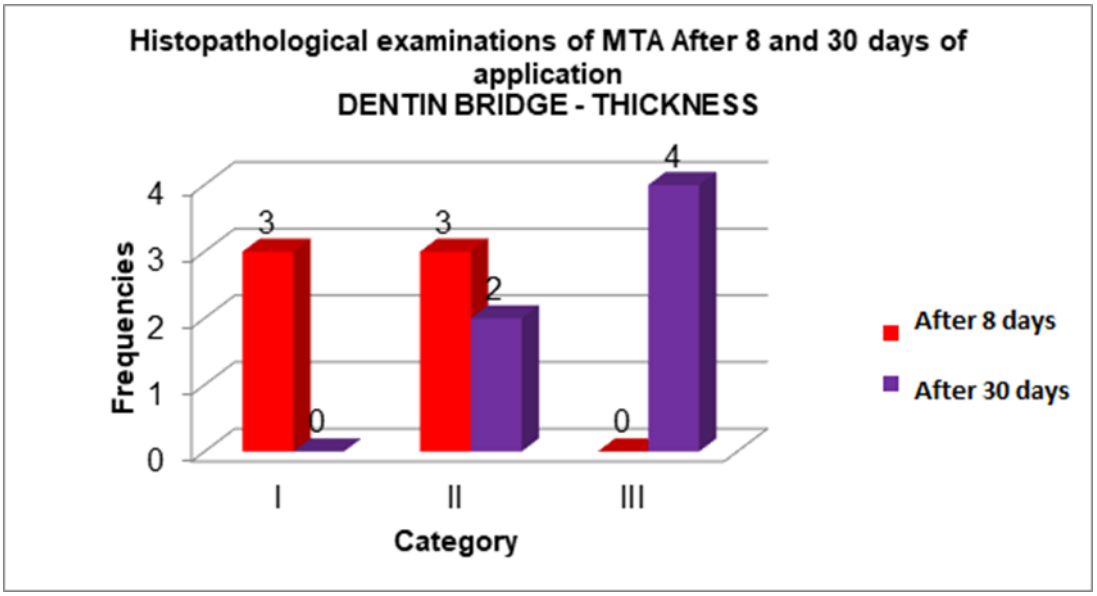


Chart 1. Histopathological Examination of MTA After 8 and 30 Days of Application – Dentin Bridge – Thickness

The ANOVA for proportions regarding the morphology of the dentin bridge after 8 and 30 days of MTA application showed that there is no statistically significant difference between categories I, II, and III, either within the groups or between the groups. This means that the observed differences in the six analyzed samples (in each group separately) are not statistically significant.

Next Characteristic Analyzed is Type of Pulp Inflammation, In the first group, 3 samples showed elements of chronic inflammation (II), while the other half of the samples had no inflammation at all (III). In the second group of analyzed teeth, only 2 samples showed chronic inflammation (II), while the other 4 showed no inflammation (III). Chart 2.

Here too, one-way ANOVA for proportions showed that there was no statistically significant difference in the number of analyzed teeth for the type of inflammation. The differences between categories I, II, and III, as well as between I-I, II-II, and III-III in both groups, were statistically insignificant.

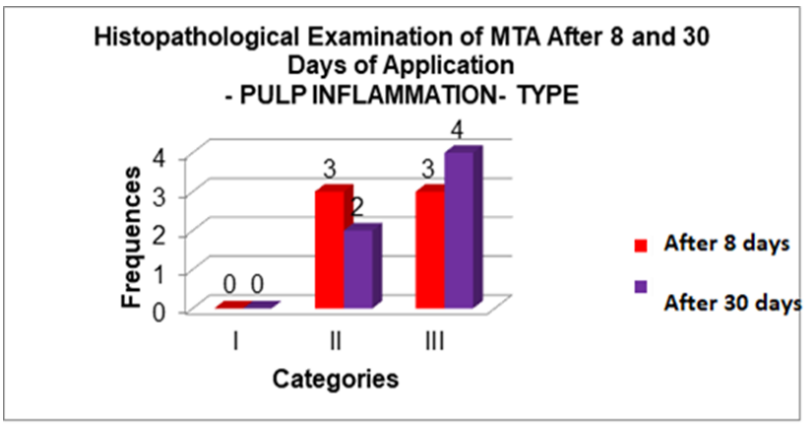


Chart 2. Histopathological Examination of MTA After 8 and 30 Days of Application – Pulp Inflammation – Type

Chart 3 presents data on the presence (or absence) of calcifications. In 2 samples from both groups, there were diffuse irregular calcifications (I), while in the remaining 4 samples, no calcification was observed (III).

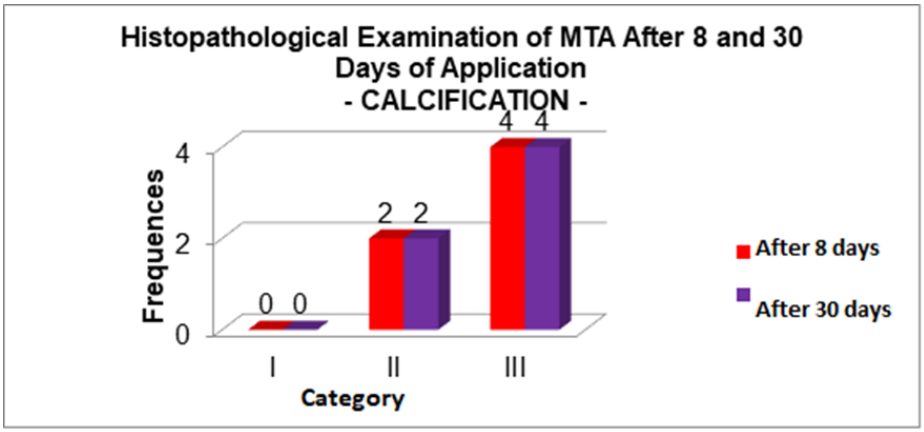


Chart 3. Histopathological Examination of MTA After 8 and 30 Days of Application – Calcification

Table 5 and **Charts 4, 5, and 6** present the distribution of samples where direct capping was performed with Biodentine. In group 3, the teeth are shown where Biodentine acted as the direct capping material 8 days prior to tooth extraction, while in group 4, the direct capping material remained for 30 days before the tooth was extracted.

		Biodentine					
		Group 1 (after 8 days)			Group 2 (after 8 days)		
Characteristics	**Category**	**I**	**II**	**III**	**I**	**II**	**III**
Dentin Bridge	**Thickness**	**3**	**3**	**0**	**0**	**2**	**4**
Pulp inflamation	***Type***	**0**	**2**	**4**	**0**	**0**	**6**
Other Histological Characteristics	***Calcification***	**0**	**0**	**6**	**0**	**0**	**6**

Table 5. Distribution of Samples Included in Histopathological Examinations Where Biodentine was Applied as a Direct Capping Agent via Pulp Exposure

In all 6 samples from the third group, a dentin bridge was formed 8 days after capping. Regarding the thickness of the dentin bridge, in 3 of the samples from the third group, the thickness of the dentin bridge is less than 0.1 mm (I), while in the other 3 samples from the same group, its thickness ranges between 0.1-0.25 mm (II). In the fourth group, in 2 of the teeth, the thickness of the dentin bridge ranges between 0.1-0.25 mm (II), while in the remaining 4, the thickness is greater than 0.25 mm (III). Chart 4. Here, the one-way analysis of variance for attributive characteristics of the observation showed no statistically significant difference.

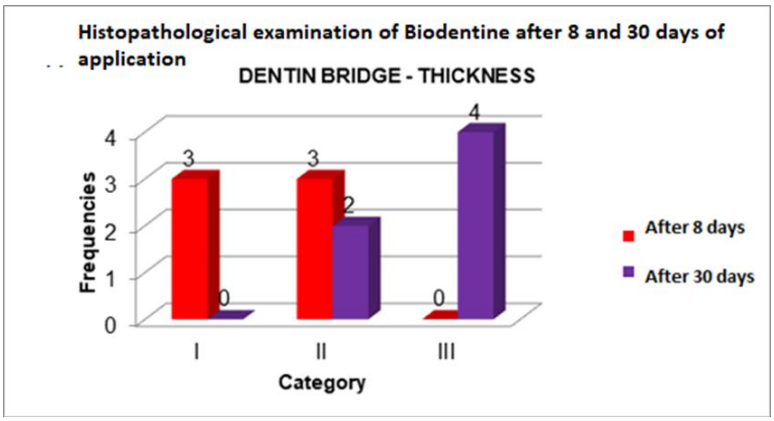


Chart 4. Histopathological Examination of Biodentine After 8 and 30 Days of Application – Dentin Bridge – Thickness

The next characteristic analyzed in the histological samples is the type of pulp inflammation. In 2 samples from the third group, moderate inflammation (II) is present, while in the remaining 4, there are no elements of either acute or chronic inflammation (III). In the fourth group, all samples show no signs of inflammation (III). **Chart 6**. Regarding this histological characteristic, there is an evident tendency of difference, but it is not statistically significant.

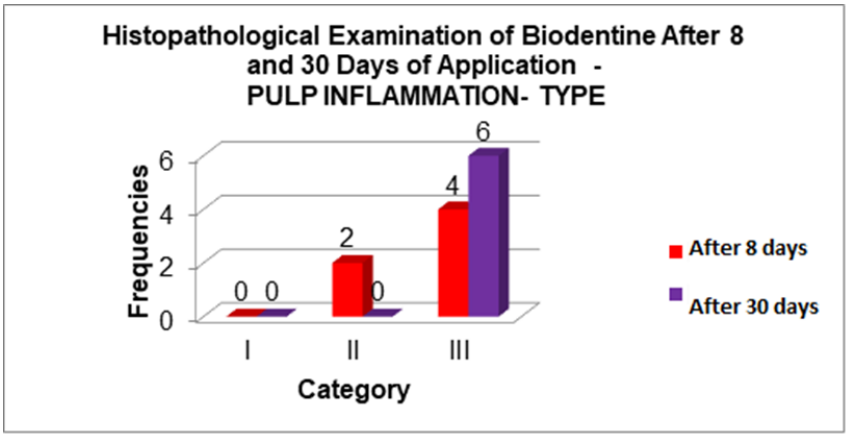


Chart 5. Histopathological Examination of Biodentine After 8 and 30 Days of Application- Pulp Inflamamtion- Type

Chart 6 presents data on the presence (or absence) of calcifications. In all 12 samples from both groups, no calcification was observed (III).

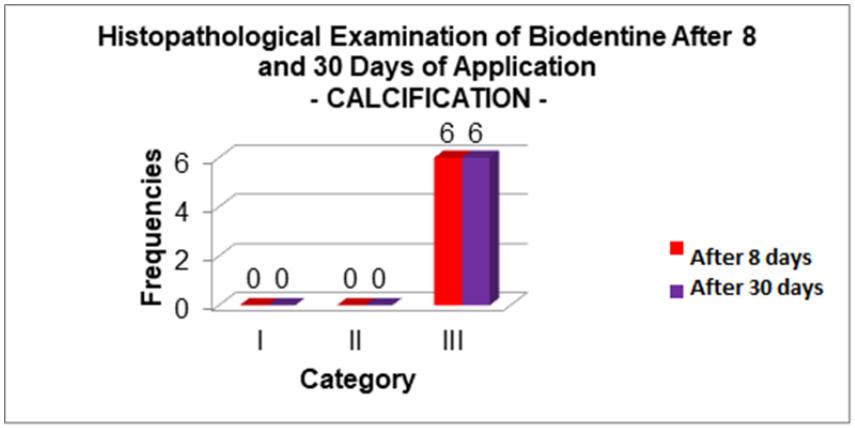
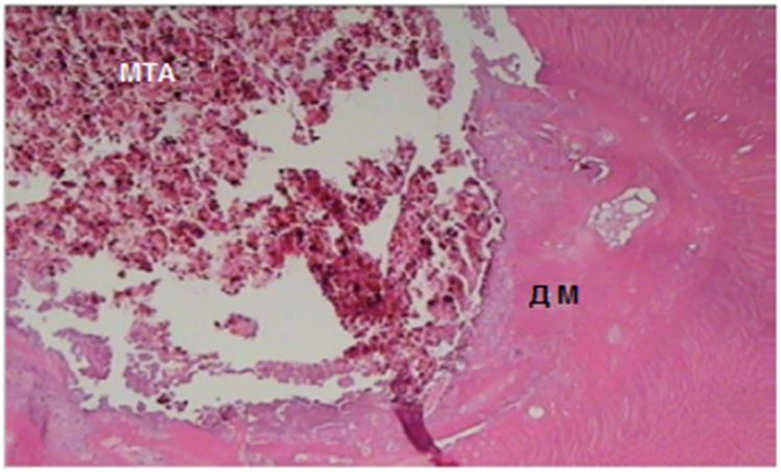
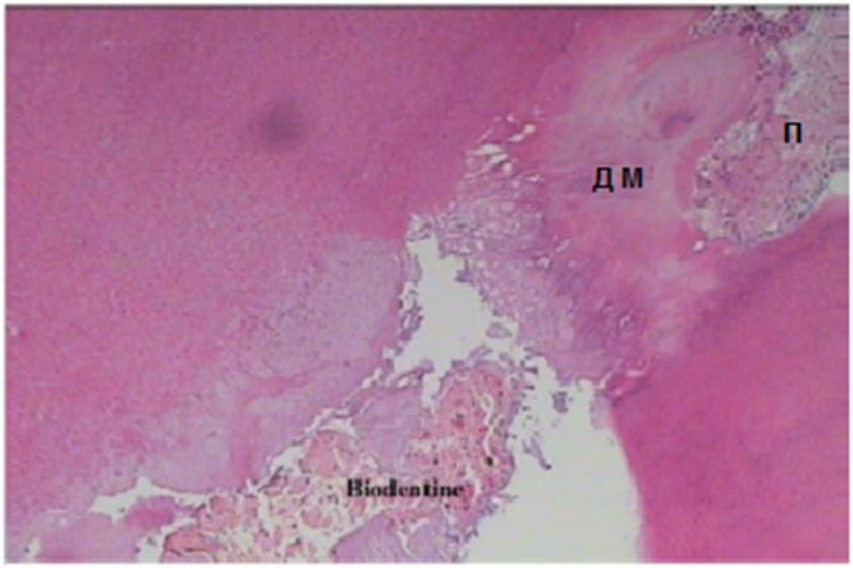


Chart 6. Histopathological Examination of Biodentine After 8 and 30 Days of Application - Calcification



Picture 2. 30 days after the application of MTA as a direct pulp capping material on a maxillary first molar; P - pulp; DM - dentin bridge.



Picture 3. 30 days after the application of Biodentine as a direct pulp capping material on a maxillary first molar; P - pulp; DM - dentin bridge.

IV. Discussion

In this part of the study, analyses were performed using light microscopy, comparing the results obtained from direct pulp capping with MTA and Biodentine®. The main aim of treating pulp exposures with appropriate direct pulp capping (DPC) material is to stimulate the dentinogenic potential of pulp cells (7). Therefore, we focused on the thickness of dentin bridges, the presence and intensity of inflammation, and other histological characteristics. The formation of a dentin bridge between the pulp and the DPC material can be controversial, as it can indicate healing, but it could also be a reaction to irritation (8, 9, 7).

Our results align with those obtained by Nowicka et al. (10), where in the majority of samples analyzed, dentin bridges formed and inflammation was absent. The results clearly show that both materials used as DPC agents induce early formation of reparative dentin, as early as 8 days after application.

Laurent et al. (11) pointed out that particles of Biodentine® were found in newly formed dentin islands, and as a result of mineralization, osteodentin formed. This suggests that the physico-chemical properties of Biodentine® promote the mineralization process. Cellular proliferation and differentiation are linked to tricalcium silicate, one of the main components in Biodentine®, as well as calcium and silicon ions (12, 11, 13, 14).

Numerous studies have presented successful results from the application of MTA as a DPC material (15, 16, 17, 18, 19). In our study, the results obtained for dentin bridge formation where MTA was applied correspond with these findings.

In the literature, many studies suggest that the pulpal response after DPC largely depends on bacterial microleakage (20, 21, 22, 17, 19). It has been observed that bacteria stimulate pulpal inflammatory activity and reduce the area available for dentin bridge formation, regardless of the material used for DPC (23).

Acute pulp inflammation and necrosis were not observed in any samples. Tabarsi et al. (24), in their experimental study on dogs, indicated that necrosis was present in 22.7% of samples after direct pulp capping with MTA. The different results can be explained by the fact that in their study, MTA was applied after a pulpotomy, whereas in our study, MTA was applied directly to the pulp exposure.

After the application of Biodentine®, moderate inflammation was observed in most cases, indicating the biocompatibility of the material.

Regarding calcification, the results show that only 2 of 6 samples from the first and second groups (MTA capping) exhibited diffuse irregular calcifications, whereas no calcification was observed in the third and fourth group samples (Biodentine® capping). Norton (25) and Stoor (26) explain the calcifications present in teeth capped with an appropriate DPC material as a result of the bio-histochemical characteristics of the material and its chemical reaction with the pulp tissue. Data on the presence of diffuse calcifications are also presented by Jabbarifar (27) in his study, which analyzed various DPC materials, including MTA.

V. Conclusion

The results from histopathological analysis show that both MTA and Biodentine have a favorable effect on pulp tissue when used as direct pulp capping agents, as they demonstrate biocompatibility and induce the formation of a complete dentin bridge across the pulp exposure. The obtained results refer to direct capping of

healthy, non-inflamed pulps. In terms of the appropriate characteristics and advantages of Biodentine over MTA, such as easier handling, shorter preparation time, and no tooth discoloration, it can be concluded that Biodentine is an alternative to MTA in direct pulp capping treatment.

Summing up the results from all the analyses, it can be concluded that MTA and Biodentine are excellent alternatives when selecting materials for direct pulp capping. Their good preparation, handling, and application characteristics, along with favorable biological, mechanical, and physical properties, allow for simplification of the DPC technique and provide a better therapeutic prognosis.

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