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Clinical and Histological Analyzes of the Response of the Pulp after Its Direct Capping with Calxyl, MTA and Biodentine.

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ABSTRACT

The caries is a destructive process that leads to a progressive demineralization of the nonorganic part of the tooth, followed by enzymatic disintegration of the organic component of the teeth tissues. Having in mind that caries activity can be stopped if one stops the process of demineralization, it is clear that the treatment of caries is directed towards the elimination of etiological factors and stimulation of the dental tissue regeneration process. This is why the treatment of caries has been targeted towards re-mineralization of initial carious lesions in the recent past years, as well as towards the study of the pulp-dental complex after an application of certain medicaments. Based on these findings, the purpose of this study was to evaluate the impact of various materials for direct pulp limitation, in order to maintain the vitality of its tissue and to monitor the success of the therapy. In order to perform this research, the following materials were used: Calxyl, MTA and Biodentine, as a solution for direct capping of pulp exposure. The examinations were split in two parts: clinical research of the direct pulp limitation effectiveness using appropriate material, and experimental research that included pathological and immune his to chemical analysis.

Keywords: pulp capping, calxyl, MTA, Biodentine

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INTRODUCTION

The goals of caries treatment are aimed at the elimination of etiological factors and the stimulation of dental tissues regeneration [1, 2]. A significant progress in the treatment of carious disease can be seen in the past recent years, one that focuses on re-mineralization of initial carious lesions and on the biological behavior of the pulp-dental complex after application of certain medicaments.

After a damage of the pulp tissue has happened, the next reparatory response will be the deposition of a tertiary dental matrix of a reactive or reparatory type of dentin. The direction in which the secretion of the dental tissue will move depends on the intensity of the initial response, and the conditions that regulate the deposition of newly formed dental matrix. Generally, the reactive dentin is created by preexisting odontoblasts, and the reparative dentin it excreted by newly differentiated odontoblastic cells [3]. That's why the reparative dentinogenesis is a much more complex process than the reactive dentinogenesis, and therefore it represents a consequential process after the preparation of cavities, where the expansion of pulp tissue happens [4]. In such situations, the clinician is the one that has to decide whether to apply a direct capping solution, or continue with pulpectomy [5].

The concept of the pulp tissue vitalization treatment is based on the use of biological principles in order to maintain the vitality of the pulp. Nowadays, the research is aimed at finding a material that is bioactive and that can constantly stimulate the cell reparatory mechanisms, in order to form biologically stable reparatory dental bridge.

Clinicians use many materials and techniques for direct capping, including $Ca(OH)_2$, hydrophilic resins, resin modified glass-ionomer cements, tricalcium phosphates, and lately the mineral trioxide aggregate (MTA), as well as the bioactive silicate cement Biodentine.

Based on these findings, the aim of this study is the analysis and monitoring of the success rate at sanitized pulp exposures after application of different materials for pulp capping (Biodentine, MTA and $CaOH_2$), the follow up of the subjective symptoms of patients after the application of medicaments in several sequential treatments, and the histopathological monitoring of the healthy human dental pulp response after the application of Biodentine and MTA directly on the exposed part of the pulp.

MATERIAL AND METHODS

We've used the following materials for the research: Calxyl, MTA and Biodentine. The type of the material that we've tested, their factory name, manufacturer and chemical compositions are given in Table 1.

Table 1: Materials used in the study

Material	Manufacturer	Chemical structure
Calxyl	*OCO* PRÄPARATE®	Calcium hydroxide, Barium sulphate,
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

The tests were carried out on two levels:

- Clinical tests
- Pathohistological tests

Clinical examination

The clinical part of the examination was conducted at the Clinic for Conservative dentistry and Endodontics which is part of the Faculty of Dentistry in Skopje. The group of examined patients was comprised

of people aged 18 to 40 years, of both sexes, that had shown indications for application of direct capping materials. During their examination it was determined that they meet the following criteria for their inclusion in the study:

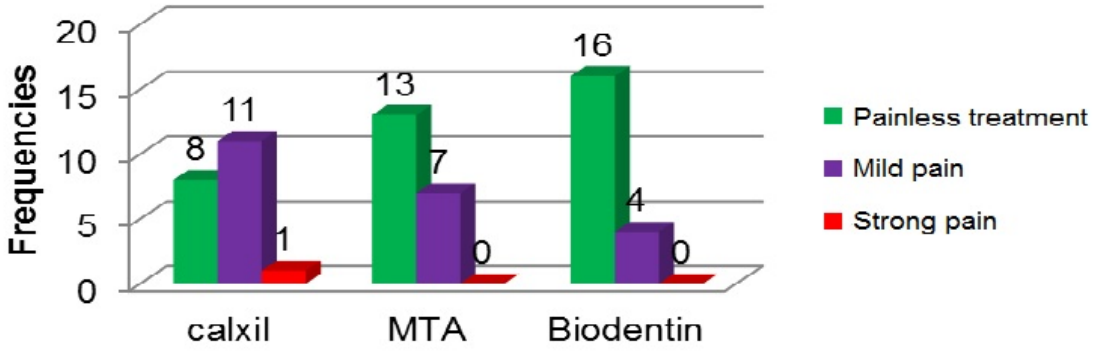
• The pulp exposure was of small dimension
• The teeth were vital, without presence of symptoms that indicate a pulp inflammation
• The location of the exposure allowed direct contact of the material with vital pulp
• The absence of periapical pathosis was ascertained radiologicaly
• Prompt control of the haemorrhage of exposed part of the pulp was established
• In the personal anamnesis of the respondents, there was absence of data on eventual allergy or hypersensitivity to drugs or other medicaments

This part of the examination covered 60 teeth, which we've divided into 3 groups (Table 2). In the first 20, the pulp exposure was covered with Calxyl (group 1), the other 20 with MTA (group 2), and at the remaining 20 teeth the pulp was directly coated using Biodentine (group 3).

Table 2: Distribution of samples involved in clinical tests where direct capping material was applied throughout the pulp exposure. a. Calxyl, b. MTA, c. Biodentine

	a. First groupb. Second group в. Third group							
	Calxyl			**MTA**			**Biodentine**	
Type of tooth	**N**	**%**		**N**	**%**		**N**	**%**
Incisors	0	0		4	20		2	10
Canines	3	15		2	10		0	0
Premolars	10	50		8	40		8	40
Molars	7	35		6	30		10	50
Summary	20	100		20	100		20	100

Graph 1: Subjective feelings at patients in terms of pain



We've begun the procedure by administering an anesthesia, followed by a cavity preparation and careful and thorough excavation of carious tissue. Upon the exposure of the pulp tissue, we've performed washing of entire cavity with physiological solution.

Then we've performed a hemostasis using a sterile tampon that we let it stay for 3-4 minutes until full hemostasis was reached. We've applied the appropriate material over the exposed part of the pulp (group 1 and 2), according to the manufacturers recommendations, and we've filled the cavities with glass-ionomer cement. In the third group, after we've prepared the capsule with Biodentine, we have applied the capping material directly on the pulp and thus we filled the entire cavity.

Exactly 5 drops of liquid is applied into the powder within the capsule, than the capsule is closed and placed in the amalgamator in order to be mixed for 30 seconds at a speed of 4000-4200 rotations per minute. (Fig.2)

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Figure 2: Preparation of Biodentine according to the manufacturer's recommendations

In the final phase, the cavities of all patients were filled in with definitive cavity fillings (glass-ionomer cements or composites) depending on the therapist assessment. We performed clinical and radiological evaluation of patients of all the three groups, after 8 and again after 30 days of the material application. That way we could've followed the vitality of teeth, the symptoms of patients present at the time of evaluation, and to radiologicaly determine whether reparatory dentin was formed. The findings were recorded in the questionnaire. After their rehabilitation, patients were asked questions related to previous experience with dentists, the treatment impressions they had, as well and the presence and intensity of pain during the treatment. All the data was recorded and analyzed separately in a previously prepared questionnaire.

Pathohistological analysis

The pathohistological analysis was conducted at the Institute of Pathohistology in Skopje. Teeth involved in this study had shown an indication for extraction out of orthodontic reasons. Selection of teeth from patients was done using the following criteria: age of patients was between 18 and 30 years old, with absence of systematic diseases among all of them. These teeth had clinically normal pulp, without the presence of caries, no restoration and without periodontal changes.

These analyzes were performed over 24 teeth, and depending on the material used for direct capping and duration of it, they were divided into four groups:

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

Two intact teeth were selected as a negative control group, without any pulp exposure and on which we didn't perform any direct capping.

These patients had previously signed a consent after they have been explained the purpose for which the extracted teeth would be used.

We've applied local anesthesia to all the patients prior to the beginning of the procedure, followed by preparation of the cavities of 1st class. Then sterile steel burs were used to perform trepanning. Once the hemorrhage was controlled using sterile cotton buds, direct capping material not thicker than 1.5 mm was applied over the pulp exposure (MTA - in group 1 and 2, and Biodentine - in groups 3 and 4). The cavities were immediately closed with Chemfil - glass ionomer (Dentsply) and composite material SYNERGY®D6 (COLTENE®).

After 8 days, teeth from group 1 and 3 were extracted, and after 30 days also teeth of group 2 and 4. Then we've cut these teeth longitudinally and we've immersed them in a solution for fast dentin demineralization Osteomol, where they have remained between 3 and 5 days, depending on whether they

were ready for tissue cutting in the size of 5 µm and painted by the Brown-Bren or Hemalaun-eosin method. After this, they were analyzed using a light microscope at different zooming.

All the data that was collected of interest to the study preparation, was statistically processed using the following statistical methods:

- These statistical series are displayed in spreadsheets and charts according to all the defined variables;
- Testing of the significance of differences among two arithmetic environments or proportions at dependent samples (tested in groups), was made with Student-ob t-test, and if it came to an irregular distribution, using non-parametric Wilcoxon test of equivalent pairs;
- Testing of the significance of differences among two arithmetic environments or proportions in independent samples was done using Student-ob t-test, and if it came to an irregular distribution, using non-parametric Mann Whitney U test;
- Testing of the significance of differences among three or more arithmetic environments, and between three and more ranks, was done by Analysis of variance (ANOVA)

RESULTS

Clinical tests

The clinical part of the study shows the results obtained by processing or statistical analysis of the data needed to achieve previously set goals. The study included 60 teeth that were divided into 3 groups. In the first 20 the pulp exposure was coated with Calxyl (group 1), the other 20 with MTA (group 2), and the remaining 20 teeth, we done direct pulp capping using Biodentine (group 3).

The analysis of **subjective feeling** at patients treated with different materials for direct capping, has shown the following results: (Table 4, Graph 1)

Table 3: Distribution of samples involved in these patohistological trials where direct capping solution was applied over the pulp exposure. a. MTA b. Biodentine

	a. First groupb. Second group			
	MTA		***Biodentine***	
Type of tooth	**N After 8 days**	**N After30 days**	**N After 8 days**	**N After 30 days**
premolars	1	3	2	4
molars	5	3	4	2
Summary	**6**	**6**	**6**	**6**

Table 4: Subjective feelings at patient in terms of pain

Subjective feeling at patients regarding pain	Calxil	MTA	Biodentine
	N	N	N
Painless treatment	8	13	16
Mild pain	11	7	4
Strong pain	1	0	0
Total	20	20	20

The double-factored ANOVA performed for attributive characteristics observation had shown the following: for Factor A = 8,23, DF = 2, p <0,01, and factor B = 17,09, DF = 2, p <0,01, where Factor A refers to groups of tested materials and factor B refers to the subjective feeling of the patients in terms of pain intensity. There is a difference regarding grouping and regarding the subjective feeling of the pain intensity. According to the factor A, the ANOVA test has shown that the statistical difference is highly significant and that the best distribution with the highest number of patients with painless treatment exists at those treated with Biodentine. More detailed analyzes performed with LSD tests had shown an existence of a highly significant statistical difference between patients treated with Biodentine and those treated with Calxil. The difference

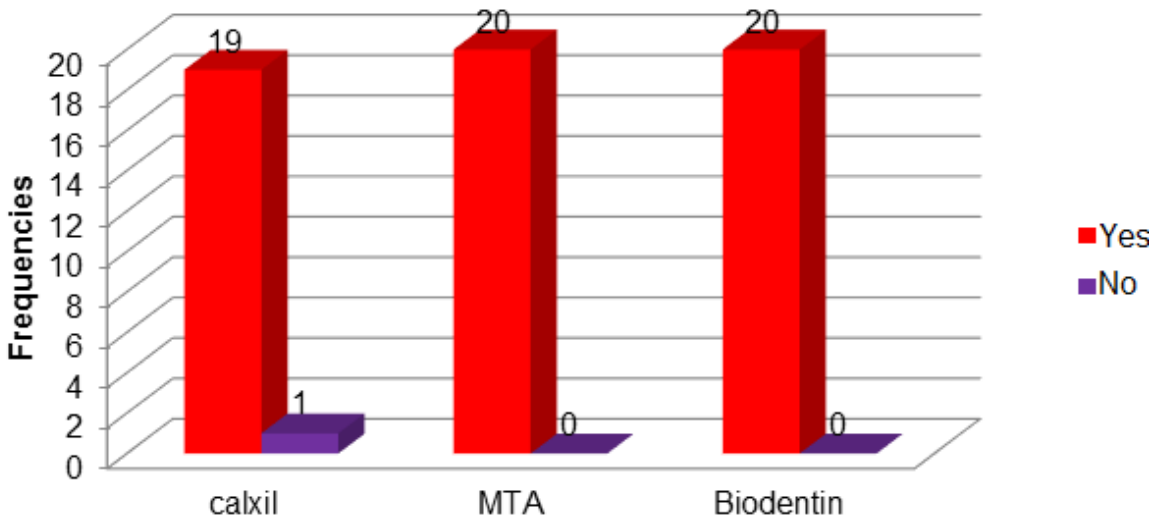
that was noticed among subjects treated with Biodentine and those with MTA was statistically significant, same as between patients treated with Calxil and MTA. According to these analyzes the highest number of patients that experienced no pain during the treatment were found in subjects treated with Biodentine. After the B factor, the ANOVA test has shown statistically highest significant difference and dominance among patients that didn't felt pain and those that had mild and severe pain. It indicates that patients experiencing no pain dominate.

The effectiveness of treatment with direct capping (DC) was analyzed by evaluation of the teeth vitality and the occurrence of spontaneous pain, after a certain period of observation (8 and 30 days)

Regarding the vitality of teeth that was examined 8 days after the application of Calxil, only one tooth out of 20 that were examined was non-vital, and 3 patients of the same group have suffered a spontaneous pain.

The two-way ANOVA for attributive characteristics of observation that were performed 8 days after the application of the direct capping, have shown that in terms of vitality and spontaneous pain, there wasn't any statistically significant difference between the different materials used for direct capping. It means that all the materials applied have shown dominance of positive effects after 8 days of their use. (Table 5, Graphs 2a, 2b).

Graph 2a: Evaluation of vitality 8 days after the application of the DC material



Graph 2b: Evaluation of spontaneous pain 8 days after application of DC material

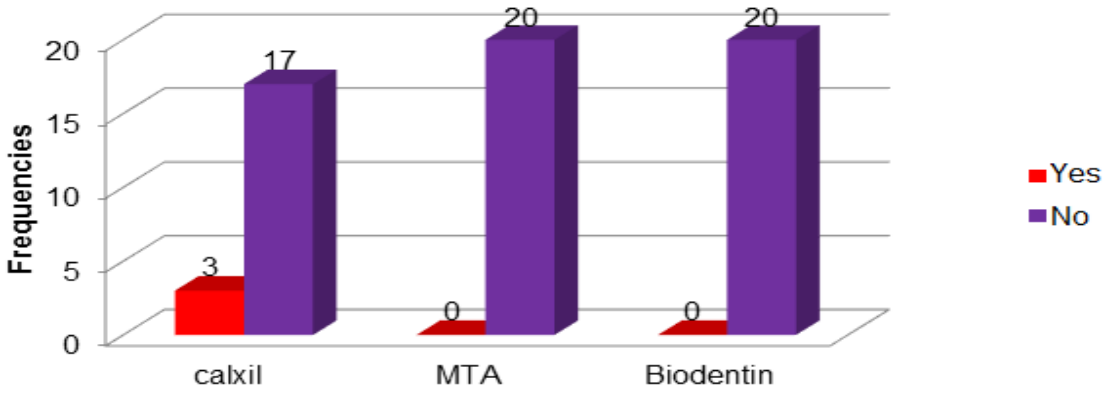


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Table 5: DC treatment effectiveness and evaluation after 8 days of application.

Direct capping treatment efficacy and evaluation after 8 days of material application	Calxil		MTA		Biodentine	
	N		N		N	
	yes	no	yes	no	yes	no
Vitality	19	1	20	0	20	0
Spontaneous pain	317		0	20	0	20
Total	20		20		20	

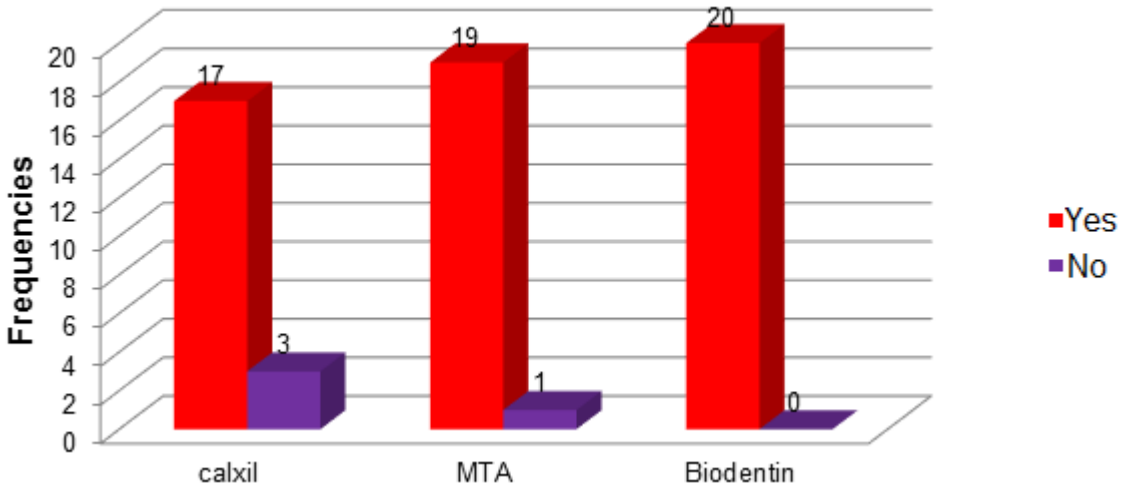
The efficacy of the treatment with direct capping using three different materials, the evaluation of the vitality, the spontaneous pain and presence of caries in teeth of patients after 30 days of direct capping treatment, has shown that in the group of patients treated with Calxil there were 3 non vital teeth found, and a spontaneous pain has occurred with a patient. In the group where capping was performed using MTA, it was noticed that after 30 days of the treatment there was only one non vital tooth found.

The ANOVA tests that were performed have shown no statistically significant difference between the different materials for direct capping. It means that after 30 days of vitality and spontaneous pain evaluation, the positive effects are statistically dominant within all the materials. (Table 6, Graphs 3a, 3b).

Table 6: The efficacy of DC treatment and evaluation after 30 days of the DC material application.

DC treatment efficacy and evaluation after 30 days from the DC material application	Calxil		MTA		Biodentine	
	N		N		N	
	yes	no	yes	no	yes	no
Vitality	173		191		20	0
Spontaneous pain	119		0	20	0	20
Presence of caries	0	20	0	20	0	20
Total	20		20		20	

Graph 3a: Evaluation of vitality 30 days after the application of the DC material



Graph 3b: Evaluation of spontaneous pain 30 days after application of DC material Graph9: Pathohistological tests of Biodentine use after 8 and after 30 days of its application - Odontoblastic layer - I (bad) - No odontoblasts, II (intermediate) - Presence of odontoblasts, III (best) - Palisades set odontoblasts

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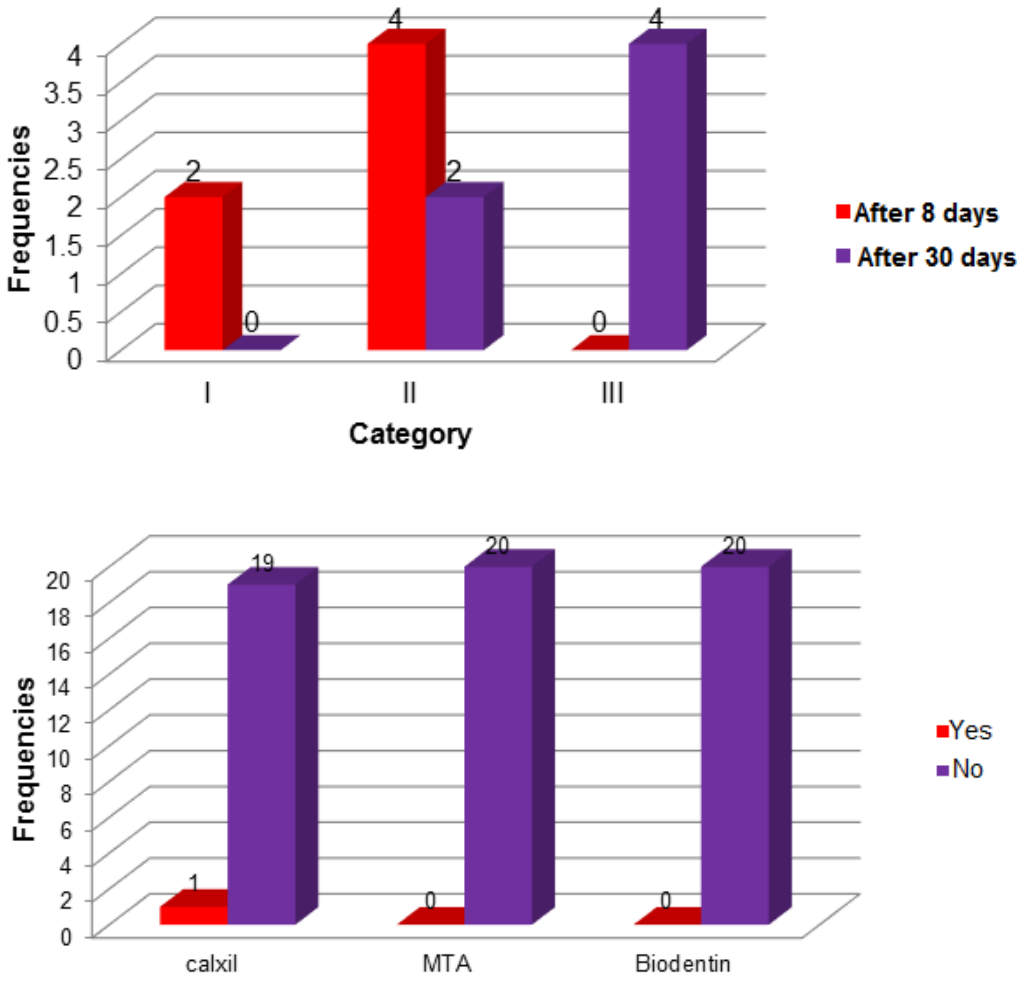
Graph 9: Pathohistological tests of Biodentine use after 8 and after 30 days of its application - Odontoblastic layer - I (bad) - No odontoblasts, II (intermediate) - Presence of odontoblasts, III (best) - Palisades set odontoblasts



Pathohistological analysis

This part of the research shows the results of the pathohistological analyzes of 12 samples where direct capping was done using MTA, and 12 samples for which Biodentine was used as a material for direct capping. In order to monitor and analyze multiple features of the histological preparations at each of the samples, the results are presented in separate tables and graphs for samples of the MTA group, and some tables and graphs present data obtained from analysis of teeth covered with Biodentine. Table 7 and Graphs 4, 5 and 6 present the distribution of the samples where direct capping was done using MTA. Group 1 represents teeth where MTA is the applied solution for direct capping 8 days prior to the extraction of the tooth, while in Group 2 the solution for direct capping remains applied 30 days before the tooth is to be extracted. Table 8 and the diagrams 7, 8 and 9 present the distribution of the samples where direct capping was done using Biodentine Group 3 consists of teeth where Biodentine acts as a direct capping material for 8 days prior to the extraction of the tooth, and Group 4 presents a situation where this solution for direct capping remains for 30 days before the tooth to be extracted.

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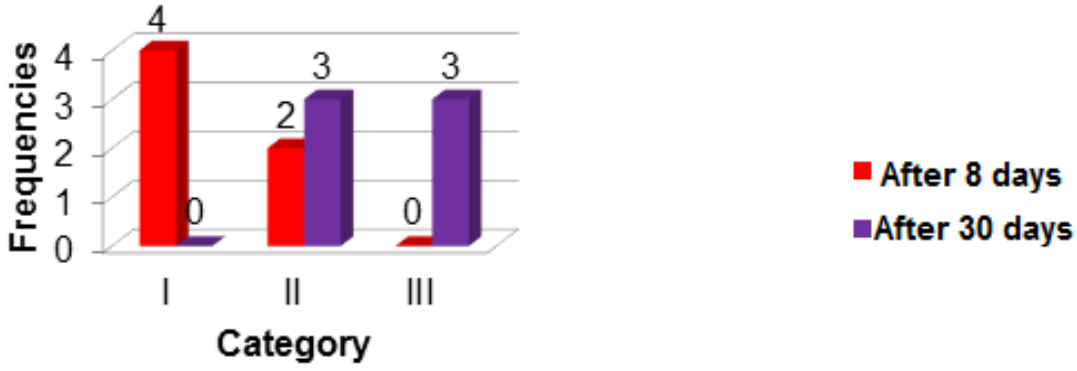
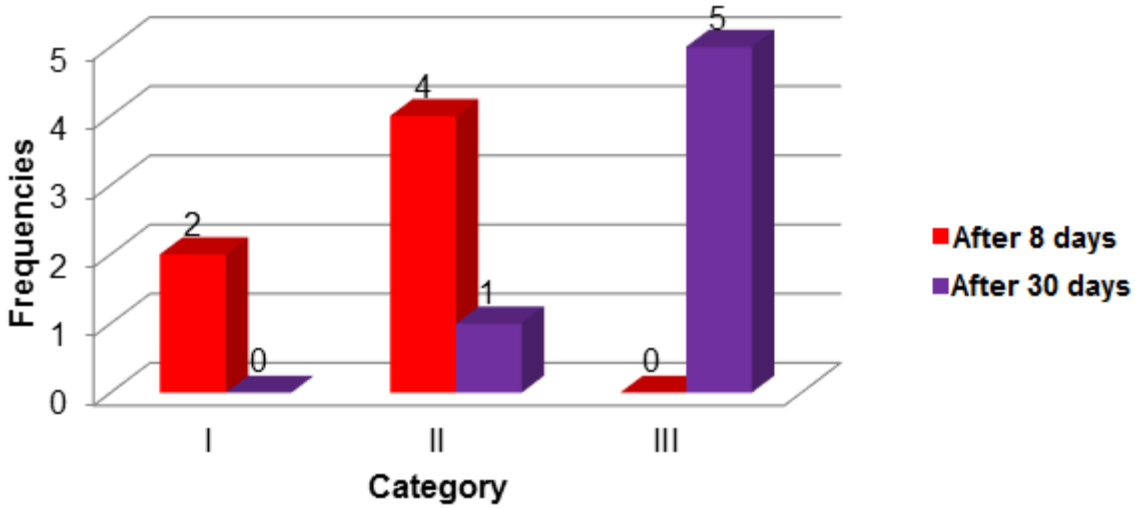
Table 7: Distribution of samples involved in pathohistological tests in which a direct capping material MTA was applied throughout the pulp exposure

		MTA					
		Group 1 (after 8 days)			Group 2 (after 30 days)		
Features	Category	I	II	III	I	II	III
Dental bridge	*Morphology*	4	2	0	0	3	3
Pulp inflammation	*Type*	0	2	4	0	1	5
	Intensity	0	2	4	0	1	5
Other histological features	*odontoblastic layer*	2	4	0	0	3	3

Graph 4: Pathohistological tests of using MTA after 8 and after 30days of its application - *Dental Bridge - morphology* - I (bad) - No presence of dental tubules, II (intermediate) - Irregular type of dental tubules, III (best) - Regular dental tubules



Graph 5: Pathohistological tests of using Biodentine after 8 and after 30 days of its application - *Dental Bridge - morphology* - I (bad) - No presence of dental tubules, II (intermediate) – Irregular type of dental tubules, III (best) - Regular dental tubules



Graph 6: Pathohistological tests of MTA after 8 and after 30 days of its application - *Pulp inflammation - the type and intensity* - I (bad) - Acute and chronic severe inflammation, II (intermediate) - Chronic mild inflammation, III (best) - No inflammation

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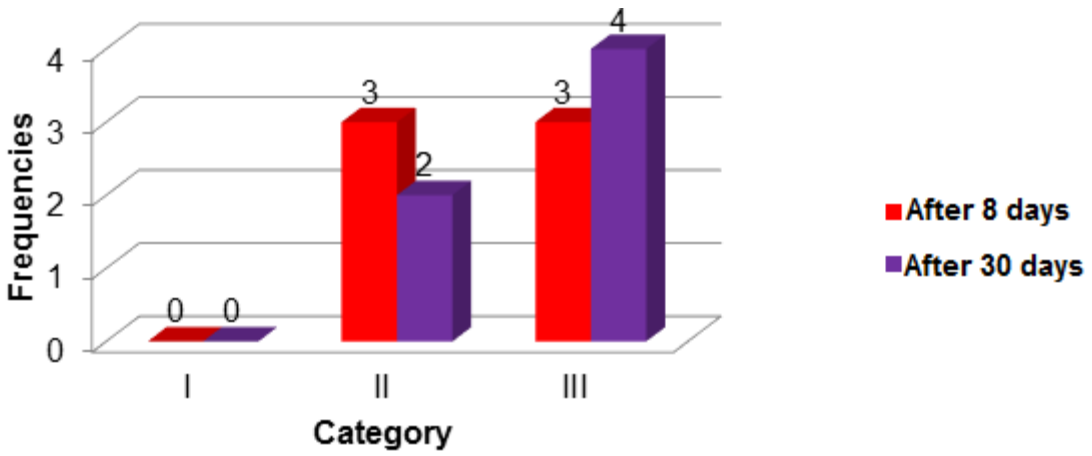

Table 8: Distribution of samples involved in pathohistological tests in which a direct capping material Biodentine was applied throughout the pulp exposure

		Biodentine					
		Group 1 (after 8 days)			Group 2 (after 30 days)		
Features	**Category**	**I**	**II**	**III**	**I**	**II**	**III**
Dental bridge	***Morphology***	**2**	**4**	**0**	**0**	**1**	**5**
Pulp inflammation	***Type***	**0**	**0**	**6**	**0**	**0**	**6**

In terms of **morphology**, a dental bridge was formed in all six samples of the first group after 8 days of the capping. Morphologically, in 4 of them there aren't any dental tubules present (I), and the remaining two teeth formed irregular types of dental tubules (II). Thirty days after the application of MTA (second group), a formation of irregular types of dental tubules has occurred at 3 of the teeth (II), and the dentin at the remaining 3 teeth was consisted of regular dental tubules (III). (Graph 4.)

In the six samples from the third group a dental bridge was formed after 8 days of the capping. Morphologically in two of them, there isn't a presence of dental tubules in the dentin (I), and the remaining 4 teeth formed irregular types of dental tubules (II). Thirty days after the application of Biodentine (fourth group), only one of the teeth formed irregular type of dental tubules (II), and the dentin at the remaining 5 was consisted of regular dental tubules (III). (Graph 5.)

The next feature that was analyzed using these histological preparations is the **pulp inflammation** - the type and the intensity. In three samples of the first group we found presence of moderate intensity chronic inflammation elements (II), and with the other half of the samples, inflammation is not present at all (III). In the second group of examined teeth, there were only two of them where we saw presence of chronic inflammation with moderate intensity (II), and the other four were without inflammation (III). Chart 6th

It is also here that the single-factored ANOVA for proportions has shown that there isn't any statistically significant difference with this number of analyzed teeth, not for the type nor for the intensity of the inflammation. The difference between the categories I, II and III and particularly between I - I, including II - II and between the III - III in both of the groups, is statistically is insignificant.

There was presence of moderate inflammation in two of the samples from the third group, (II), and there weren't any elements of an acute or chronic inflammation nature present with the remaining 4 (III). In the fourth group of all samples not present in inflammation (III). (Graph 7.) In terms of this histological feature, there's an evidence of the tendency for existence of differences, but this isn't statistically significant because of the size of the samples.

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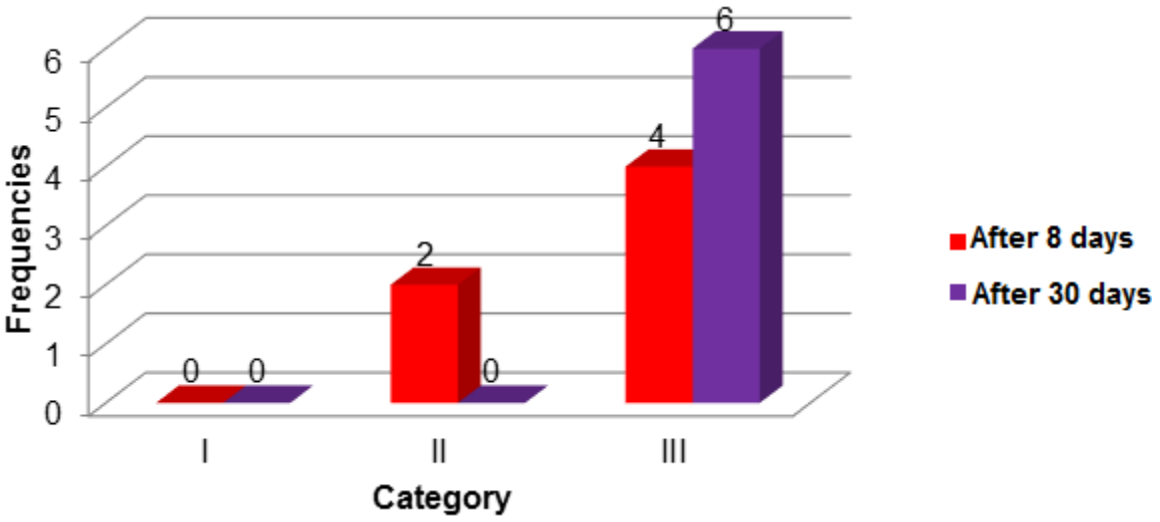
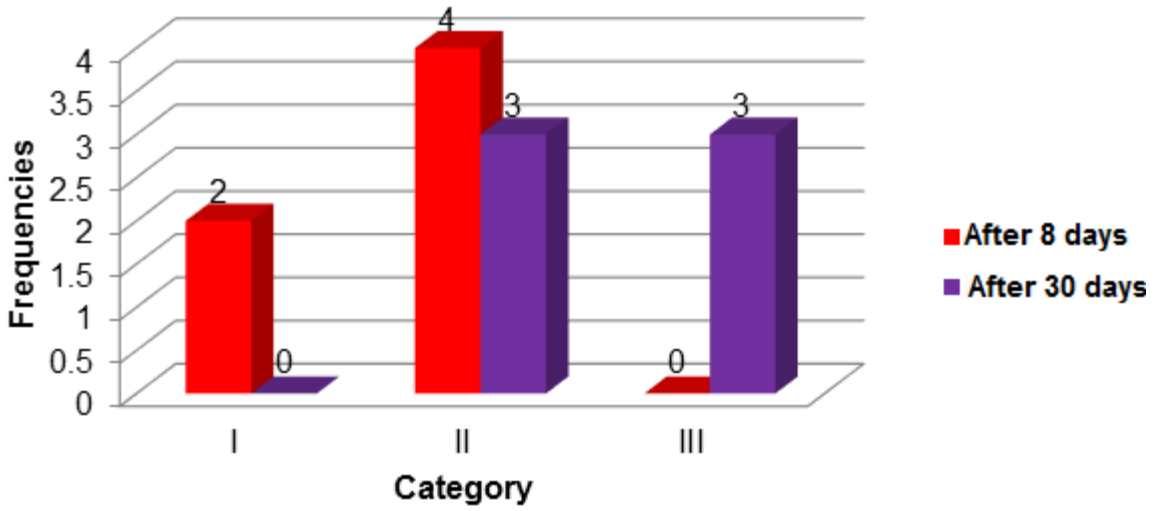
Graph 7: Pathohistological tests of Biodentine after 8 and after 30 days of its application - *Pulp inflammation type* - I (bad) - Acute and chronic severe inflammation, II (intermediate) - Chronic mild inflammation, III (best) - No inflammation.



The last analyzed histological feature shows presence of **odontoblastic layer**. In the first group there are odontoblasts in 4 out of 6 samples (II), and in the second group there are odontoblastic layers in all of the six teeth, of which three have them set as palisade (III). (Graph 8.) Tests made using single-factored ANOVA for attributive features of observation have shown that even according to these features there wasn't any statistically significant difference among the three histological features between categories I, II and III, nor between the two groups separately. It is believed that this is due to the fact that it is a small statistical sample.

Graph 8: Pathohistological tests of MTA use after 8 and after 30 days of its application - *Odontoblastic layer* - I (bad) - No odontoblasts, II (intermediate) - Presence of odontoblasts, III (best) - Palisades set odontoblasts



Odontoblasts were found at 4 out of 6 samples from the third group (II), and the fourth group resulted with presence of odontoblastic layer at all six teeth, out of which 4 has shown palisade setting of odontoblasts in their case (III). Chart 9th.

Tests that were made using single-factored ANOVA for attributive features of observation, have also shown that regarding these features there isn't any statistically significant difference among the three

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histological features of the categories I, II and III, nor between the two groups separately. It is believed that this is due to the fact that it is a small statistical sample.

Figure 5, 6, 7 show part of the histological preparations of cavities where an appropriate material for direct capping was applied through the pulp exposure.

Figure 5 a, b: Eight days after the application of MTA as a solution for direct capping of the maxillary first molar; П - pulp; DM - Dentinal bridge

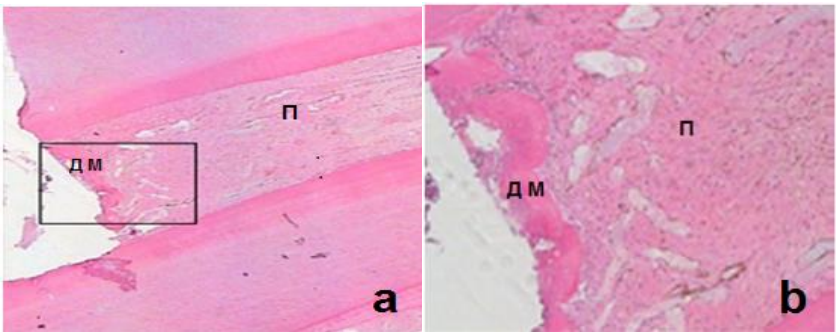


Figure 6: Eight days after the application of Biodentine as a solution for direct capping of the second maxillary molar; П - pulp; DM - dentinal bridge

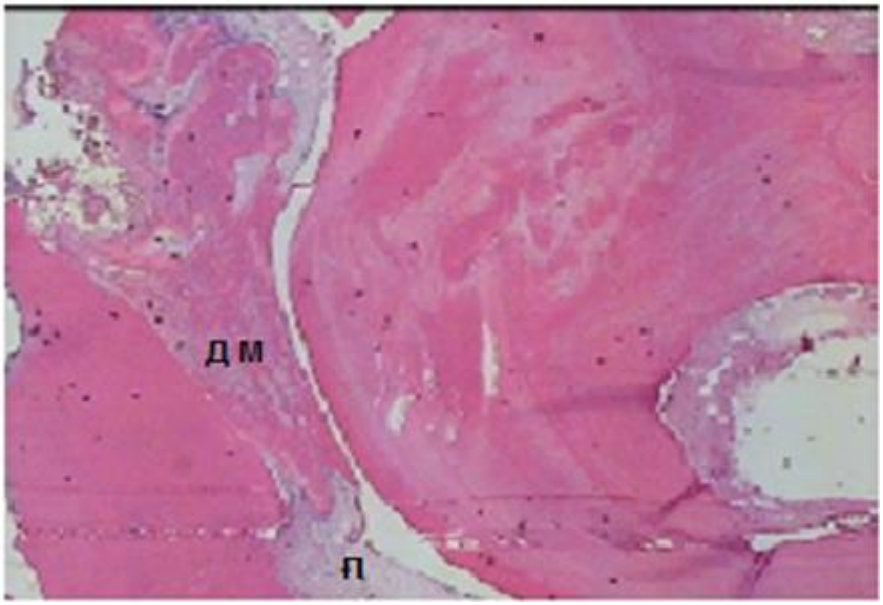
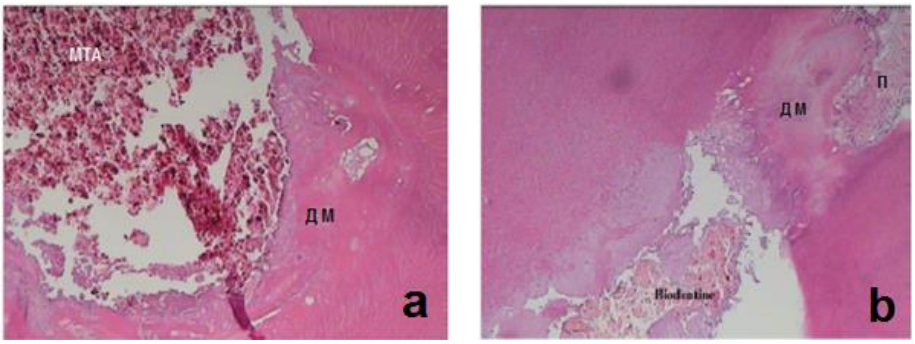


Figure 7: Thirty days after the application of MTA (a) and Biodentine (b) as materials for direct capping of the maxillary molars; П - pulp; DM – dentinal bridge



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DISCUSSION

Dental researches focus on strategies, techniques and materials that are applied if there is even a minimum indication for preservation of the pulp tissue vitality [6]. Having in mind the importance of the pulp tissue vitality, clinicians focus on discovering materials for direct capping that will have an adhesive features, that will prevent the bacterial flow, be bioactive and ones that will encourage faster pulp healing, initiating reparatory dentinogenesis. There are many factors that play a role in the prognosis of the direct capping treatment [7], the absence of inflammation, infection control, and biocompatibility of the material used, are considered key factors for the outcome of the therapy, and we still shouldn't abandon the cooperativeness with the patients and their regular checkups [8].

Data obtained during these clinical tests of patients referring to the subjective feeling in regards to the direct capping treatment using three different materials - Calxil, MTA and Biodentine, indicate that in 80% of the patients treated with Biodentine, 65% of patients treated with MTA and 40% of them in the case where direct capping was done using Calxil, the treatment turned painless, unlike for the remaining patients in all the three groups that felt mild pain (Table 4 and Figure 1). The effectiveness of the direct capping treatment in this research was assessed by evaluating the vitality of teeth, and on the occurrence of spontaneous pain after a certain period of observation (8 and 30 days). In relation to the vitality of teeth examined after 8 days of the application of Calxil, only 5% of the examined teeth were non vital, and for 15% of this group of patients teeth there was an evidence of a spontaneous pain. In the other two groups that were examined, where direct capping was performed using MTA and Biodentine for a period of 8 days, all the teeth were vital, without spontaneous pain and presence of caries. It means that positive effects dominate at all the materials that were used as a treatment for 8 days. (Table 5, graphs 2a, 2b) In regards to the calcium hydroxide and despite the fact that it has been recognized as a "gold standard" for direct and indirect capping, there are lots of failures that are attributed to it, mainly due to the weak bondage with the dentin, the weaker mechanical connection and chemical instability [9].

Figure 1: Calxyl OCO PRÄPARATE®; MTA Dentsply; BiodentineSeptodont

Figure 3 (a, b, c): Pulp exposure of tooth 15, where we can see an application of Biodentine as a direct capping material

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Figure 4 (a): Radiological display of tooth 36, seen with caries presence and filled with older material. (b). Radiological display of the same tooth, 30 days after the application of Biodentine.

Al-Hiyasat AS [10] observes in his radiological study that complications of the pulp in the case when the cavities were coated with calcium hydroxide when there's dissolvent of the material under the composite loads.

Same results were found thirty days after direct capping with these three different materials was done, just like in the case of the post 8 day evaluation of these same parameters (vitality, spontaneous pain and presence of caries) (Table 6 graphs 3a, 3b)

There were analyzes performed within the histological part of this research using a light microscope, that compare the results obtained by direct capping of the pulp with MTA and Biodentine®. In fact, the goal of the pulp exposure treatment with appropriate materials for direct capping, is to stimulate the dental potential of the pulp cells [11]. That's why we here were focused on the morphology of the dental re-bridging, the presence and the intensity of the inflammation and other histological features. Overall data from the results obtained testing these four groups, indicate that the iatrogenic pulp defects that were treated with both agents were generally without any or with only mild pulp inflammation, and were covered with compact dental bridges. The formation of a dental re-bridging on the surface between the pulp and the direct capping agent can be controversial, because it can be a sign of a recovery, and on the other hand it may be a reaction to the irritation [11, 12, 13].

Our results correlate with those obtained by Nowicka et al [14] where the majority of samples analyzed in the study has shown evidence of formation of a dental re-bridging and absence of inflammation. In the same study, the author suggests a layer of odontoblasts and cells that resemble them (odontoblastic cells), which are thought to be responsible for the formation of tubular dentin under the osteodentin.

It is also evident form the results that both the materials that we used as a direct capping material, induce early formation of a reparative dentin, 8 days after the application of direct capping. Regarding the morphology of the newly formed dentin, at the first two groups treated with MTA there's a notification that after 8 days of its application (1 gr.) at 4 of the samples there wasn't formation of dental tubules, and in the remaining 2 teeth there was formation of irregular dental tubules. Thirty days after the application (2 gr.) half of the samples had irregular, and the other half had regularly shaped dental tubules. Our results are consistent with the histological examinations done by Zarrabi MH [15].

Laurent et al. [16] indicate that particles of Biodentine® were found in newly formed dental islets and the osteodentin occurred as a result of the mineralization. This indicates that exactly the physicochemical characteristics of the Biodentine® stimulate the mineralization process. Stimulation of the cell proliferation and differentiation, is associated with the tricalcium silicate, being one of the major components of the Biodentine®, as well as with calcium and silica ions [16, 17, 18, 18, 19].

The study of Tran et al. [20] has followed the ability of Biodentine compared with the calcium hydroxide and MTA to induce pulp recovery of exposed pulp of mice. After 7 days results have shown that both MTA and Biodentine® stimulate cell proliferation and formation of mineralization zones. After a long observation, the formation of a homogenous dental bridge on the side of the pulp exposition was evident, that was leaked from the odontoblasts. The reparative dentin that was formed at samples coated with $Ca(OH)_2$ was

of porous structure, very different than the dentin that was induced by the Biodentine®. Similar to our observations, dental tubules could be clearly observed in this study too.

There wasn't any acute pulp inflammation and necrosis found in the samples. In his experimental study of dogs teeth pulp being directly coated with MTA, Tabarsi et al. [21], indicate that in 22.7% of the samples, there was a necrosis present. Different results can be explained by the fact that in this study the MTA was applied after the pulpotomy, while in our study the MTA was applied directly to the pulp exposure.

After the application of Biodentine® a moderate inflammation was noticed in most of the cases, suggesting the biocompatibility of the material.

The presence of odontoblastic layer is evident for all of the samples studied in the four groups, and the difference in the distribution of odontoblasts between examined groups is insignificant.

CONCLUSIONS

It can be concluded, based on the clinical testing, that positive effects of the treatment had dominated with more of the patients after the application of the direct capping materials. However, comparing the respondents from all the three groups, the rate of the successfully repaired pulp exposures after a period of evaluation, was greater in those cases where MTA and Biodentine were used as a materials for direct capping. Analyzing the several day evaluation of patients treated with Biodentine, MTA and $CaOH_2$, it can be found that pain was totally absent throughout the evaluation period only at participants that were treated with Biodentine. The results of the histopathology analysis show that both MTA and Biodentine have a favorable impact on the pulp tissue when used as a direct capping material, because they demonstrate biocompatibility and induce formation of a complete dental re-bridging through the pulp exposure.

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