HISTOLOGICAL EVALUATION OF INDIRECT PULP CAPPING PROCEDURES WITH CALCIUM HYDROXIDE AND MINERAL TRIOXIDE AGGREGATE

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Abstract

Objective: to obtain a histological comparison of tertiary dentin after indirect pulp capping procedures using calcium hydroxide or MTA in teeth with deep carious lesions, regarding its morphological characteristics and degree of inflammation induced in the dental pulp. We used 60 third molars with occlusal caries lesions from 30 patients of 17-26 years of age. Class I and II deep cavities were prepared and randomly restored with calcium hydroxide or MTA and glass ionomer cement. The histologic specimens were obtained at 2-4-6 weeks and the evaluation was done by the same person in a double blind manner.The results showed statistical significant differences between these capping materials regarding the formation rate and morphology of tertiary dentin induced by indirect pulp capping procedures. Conclusion: the success of this therapy is conditioned by the use of dental materials that contain or can deliver calcium hydroxide, completed with a final restoration without marginal leakage, which prevents microbial infiltration from oral cavity.

Keywords: Calcium hydroxide, MTA, dental pulp capping, tertiary dentin

Introduction

The principles that constitute the fundamentals for therapeutical concepts in dental caries are characterized by numerous modifications and intense controversy during the years in dental literature, mainly due to difficulties in reaching to the correct diagnosis and best treatment. A

profound knowledge in the histopathology of dental pulp and dentin proved to be essential in evaluating the results of clinical studies regarding indirect pulp capping with different materials (Briso et al. 2006, Chacko et al. 2006, Anderson et al. 1991).

After cavity preparation, the dentin is subjected to direct stimulation from oral cavity environment including microorganisms, that invade the dentinal tubules. Under these circumstances, the dental treatment should provide the total elimination of noxious factors and protection of dentin surface and subjacent dental pulp with materials that enhance the dentinogenetic mechanisms (Bjorndal 2002). A thorough knowledge of deep caries histomorphology proved to be essential in the evaluation of clinical results obtain after indirect pulp caping procedures using different materials in order to maintain dental pulp vitality .

During dental treatment of simple carious lesions, the protection of dentin-pulp complex is always justified and its purpose is to stop caries progression, to prevent dental pulp inflammation, to stimulate tertiary dentin formation and remineralization of uninfected demineralized dentin (Bjorndal 1995).

The purpose of our study is to conduct a histological comparison of clinical results obtained after indirect pulp capping procedures in deep carious lesions using calcium hydroxide and MTA, based on morphology of tertiary dentin and degree of inflammation induced in the dental pulp.

Material and methods

This study was conducted on 30 patients 17-26 years of age with a total of 60 third molars scheduled for extraction due to orthodontic reasons, that accepted to enter the study. All the patients, for those under 18 years of age the legal representatives, received complete information about aim, methodology and possible risks of the study and only after this procedure was completed they were asked to sign a written agreement, according to the rules of Commission of Research Etic from our university.

The following including criteria were used:

- Teeth from clinic healthy patients, without systemic diseases that could influence the dental pulp reaction to the capping material;
- Occlusal or proximal carious lesions that involved only one marginal crest;
- Carious lesion that can be easily restaured by coronal filling;
- No clinical or radiographical signs of pulp or periapical inflammation.

From the total of 60 teeth we made 2 study groups with 30 teeth each that randomly received indirect pulp capping with either Life cement (Kerr) or MTA (Dentsply) (Table 1). For the coronal filling we used light cured

glass ionomer cement Kavitan LC (Spofa). Each group was further divided in 10 teeth that were extracted after an interval of 2-4-6 weeks and prepared for histological examination, based on the following protocol:

- Storage in formalin solution 10% for 10 days;
- Decalcification with EDTA solution 10% for 4 weeks;
- Dehydration with ethilic alcohol solution 90%;
- Paraffin inclusion;
- Sections of 3-5 microns were obtain and stained with Hematoxylin-Eosin and Trichrome Szekely.

The histological evaluation was made by the same investigator and was repeated for 10% of the specimens after 4 weeks from the firs examination. Then histopathological changes of dental pulp were assessed based on the following criteria:

1 – Normal pulp, without signs of inflammation;

2 – A few inflammatory cells limited to the odontoblastic layer, without extension into the subjacent pulp tissue;

3 – Presence of inflammatory reaction of the pulp subjacent carious lesion, with hyperemia, fibrosis and moderate inflammatory infiltrate.

The tertiary dentin formation was evaluated as follows:

1- Absence of tertiary dentin

2- Islands of tertiary dentin (interrupted layer of reactive dentin)

3- Complete bridge of tertiary dentin.

The morphological aspects of tertiary dentin formed were evaluated using the following scores:

1- Unidentified structural changes
2- Random demineralization at the coronary and radicular level of the pulp
3- Dentin reaction of amorphous nature, existence of cellular inclusions
4- Dentin reaction structure similar to physiological dentin reaction, presence of dentinal tubules

Statistical analysis was based on One-way ANOVA test from GraphPad Prism program, that was used to determine the differences between study groups regarding dental pulp inflammation (code 1-3), presence of tertiary dentin (scores 1-3) and its morphology (scores 1-4) in relation with the capping material used, for a $p< 0,05$.

Tabel 1. Distribution of study groups regarding the type of cavity and capping material.

Age	Cavity Class I		Cavity Class II	
	MTA	Life	MTA	Life
17	2	2	2	1
18	2	2	1	2
19	2	2	2	2
20	2	1	1	1
21	2	2	3	1

22	2	2	2	2
23	1	1	1	-
24	1	2	3	-
25	-	1	1	-
26	2	1	-	3

Results

The procentual values calculated for the scores obtained for each study group are presented in tables 2-4. We encountered no failures, defined as spontaneous or induced pain after the treatment procedures. Statistical analysis regarding tertiary dentin formation and morphological aspects of reparative dentin showed significant differences (p<0,0001) between study groups treated with Life cement (noted by L2, L4 and L6 according to the moment of extraction) compared with MTA groups (noted as MTA2, MTA4 and MTA6).

In the groups MTA4 and MTA 6 we noted a complete tertiary dentin bridge 60% respectively 70% of the cases, compared to groups L4 and L6 where the values were 30% and 50% respectively.

In the case of tertiary dentin morphology in connection with the capping material used, in the groups MTA4 and MTA6 the aspect was similar to secondary dentin formed under physiological conditions, with dentin tubules present in 60% respectively 70% of the cases, where for the groups L4 and L6 the values were 20% and 30% respectively. The evaluation of dental pulp inflammation showed no statistical significant differences between the study groups (p=0,1997).

Tabel 2. Rate of tertiary dentin formation

Group	**Tertiary dentin formation**		
	1	**2**	**3**
L2	40%	40%	20%
L4	30%	40%	30%
L6	10%	40%	50%
MTA 2	-	60%	40%
MTA 4	10%	30%	60%
MTA 6	-	30%	70%

p<0,0001

Tabel 3. Morphological characteristics of tertiary dentin

Group	**Morphology of tertiary dentin**			
	1	**2**	**3**	**4**
L2	40%	10%	40%	10%
L4	30%	20%	30%	20%
L6	10%	30%	30%	30%
MTA 2	-	10%	20%	70%
MTA 4	10%	10%	20%	60%
MTA 6	-	10%	20%	70%

p<0,0001

Tabel 4. Evaluation of dental pulp inflammation level

Group	Level of pulpal inflammation		
	1	2	3
L2	80%	10%	10%
L4	80%	10%	10%
L6	90%	10%	-
MTA 2	80%	10%	10%
MTA 4	80%	20%	-
MTA 6	90%	10%	-

p=0,1997

The histological evaluation of specimens from the study groups according to the capping material and the study period is presented in the following figures (Fig. 1-7):

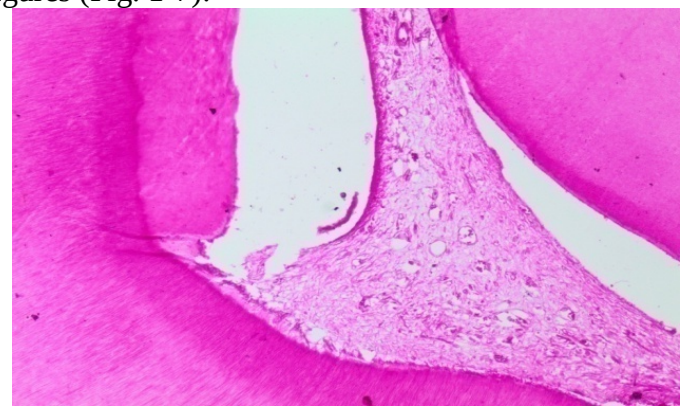

Fig. 1 Group L2. The presence of odontoblastic layer in close contact with predentin. Numerous capilares present in the pulp tissue are an expression of intense metabolic activity. The tertiary dentin is formed. (Hematoxilin – Eosin (H&E), X 20).

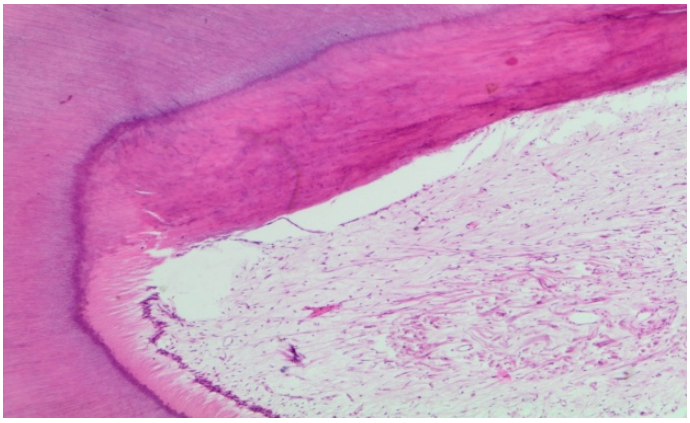

Fig.2 Group MTA 2. Pulp tissue with many cells and blood vessels, a well defined odontoblastic layer and a bridge of tertiary dentin with amorphous inclusions. (H&E, X 20).

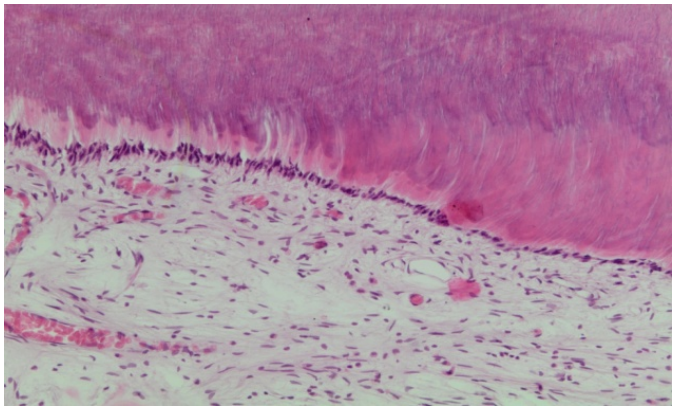

Fig.3 Group L4. A great population of cells in the dental pulp present also in the cell free zone, dilated blood vessels and a dense odontoblatic layer, beneath a thick bridge of tertiary dentin with dental tubules, similar to secondary dentin.(H&E, X 20).

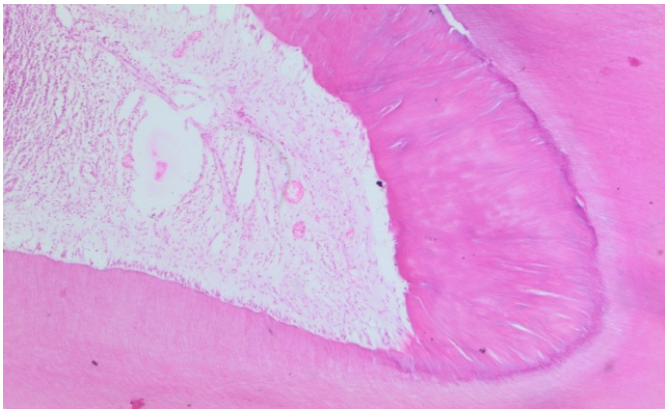

Fig. 4 Group MTA 4. Thick barrier of tertiary dentin with dentin tubules surrounding a pulp tissue free of inflammation. (H&E, X20)

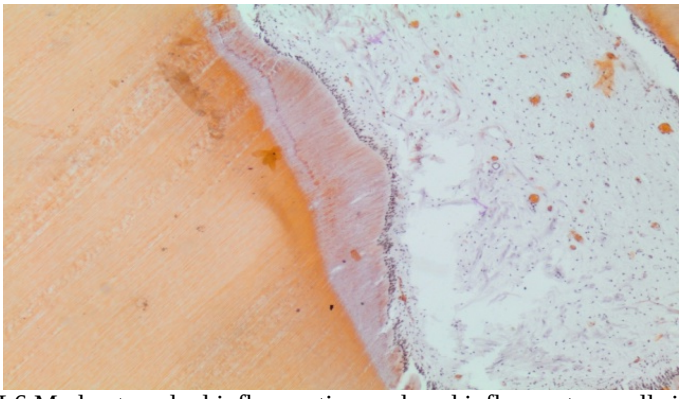

Fig. 5 Group L6 Moderate pulpal inflammation, reduced inflammatory cells infiltrate and a few dilated blood vessels. The tertiary dentin formed is rich in dentin tubules that seem to be in close connection with those of the secondary dentin. (Trichrome Szekely, X20)

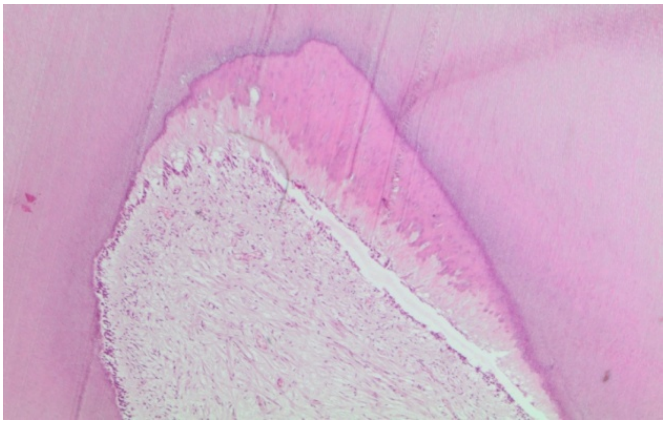

Fig. 6. Group MTA 6. The presence of the odontoblastic layer beneath a thick predentin zone and above it a barrier of tertiary dentin with dentinal tubules. The aspect is characteristic to a mild pulpal stimulation, with the preservation of the normal histologic aspect of the pulp tissue. (H&E, X 20).

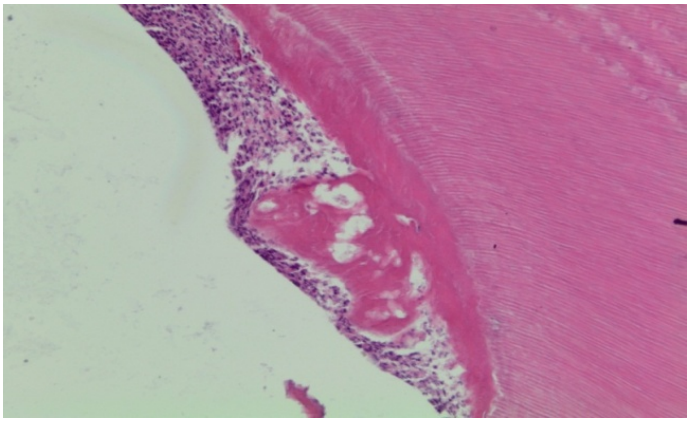

Fig.7 Group L6. Calcification in the pulp chamber with the development of a pulp stone.

Discussion

The dental materials with calcium hydroxide used in capping procedures can induce tertiary dentin formation in deep carious lesions. This process takes place only in favourable morphological and functional conditions, represented by the presence of odontoblasts and undifferentiated mesenchymal cells which are responsible of dentinogenetic function (Bjorndal 2008, Casagrande et al. 2010, Senawongse et al. 2008). These requirements are met only in young patients, as after 40-45 years of age there is an important decrease in cell population of dental pulp, accompanied by a reduction of dentinogenetic capacity.

For the teeth in our study groups, the therapy was successful in all the cases, as the patients were asymptomatic and vitality tests remained in normal limits compared to neighboring teeth. These excellent results were

explained by correct diagnosis, complete removal of decayed dentin, the use of a propper pulp capping material (Life cement or MTA) and a coronal restoration that could prevent microleakage from the oral cavity (Briso et al. 2006, Espelid et al. 1999, Steverson et al. 2002, Pashley 1991). The importance of a good coronal seal was emphasized by many experimental and clinical studies, which demonstrated that the absence of microorganisms and nutrients at the interface of dental material with subjacent dentin will assure clinical success even in the presence of demineralized dentin at the base of dental cavity (Al-Hiyasat et al. 2006, Costa et al. 2003, Snuggs et al.1993, Faraco et colab. 2001).

Estrela and colab. noted that pastes with calcium hydroxide have a better antimicrobial effect than Dycal and MTA against certain species (Enterococcus faecalis, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus) or fungi (Candida albicans). For Dycal the antibacterial effect was the least, results confirmed by Reeves et al. Similar results were published by Leye Benoist et al. who evaluated the tertiary dentin formation induced by Dycal and MTA in a group of teeth treated by indirect pulp capping and concluded that MTA gave better results. Ferracane end colab. confirmed that only dental materials that contain or may release calcium hydroxide can induce the releasing of bioactive proteins as Transforming Growing Factor 1 beta (TGF 1 beta) which are present in the dentin and released under certain condition in order to stimulate tertiary dentin formation.

The results of our study showed that Life cement represents an intense stimulating agent for tertiary dentin formation compared with MTA, due probably to the higher pH; MTA releases slowly the calcium hydroxide during setting time and therefore is considered to induce a mild stimulation.

The dental materials with calcium hydroxide demonstrated an excellent long-term success rate in deep caries therapy and therefore they have maintained an important role in modern conservative dentistry (Accorinte MLR et al, 2008). On the other hand, MTA also has the capacity to release calcium hydroxide and more, a very good dentin seal and setting in the presence of moisture (Olsson et al.2006, Furey A et al. 2010). All these allow us to conclude that today we have efficient therapeutical methods that can be used in the treatment of deep carious lesions and a carefully case selection will provide the best conditions in preserving the vitality of dental pulp.

Conclusion

Indirect pulp capping is a conservative treatment option that characterized by an excellent success rate in the treatment of deep carious

lesions. For these results, it is mandatory to use dental materials with calcium hydroxide and to avoid microleakage after coronal restoration.

The results of our study showed that formation rate and histological aspect of tertiary dentin is influenced by the material used for indirect capping, but statistically significant differences were noted only on short-term follow up periods of 2-4 weeks.

MTA proved to induce a mild stimulation in inducing tertiary dentin compared to Life cement, but the morphological characteristics of this reparative dentin were more similar to secondary physiologic dentin. Anyway, these differences between pulp capping materials were less evident after longer periods of time.

References:

Briso ALF, Rahal V, Mestrener SR, Dezan E. Biological response of pulps submitted to different capping materials. Brazilian Oral Research, 2006, 20(3): 219-225.

Chacko V, Kurikose S. Human pulp response to Mineral Trioxide Aggregate (MTA): A histologic study. J of Clin Ped Dent, 2006, 30(3): 203-209.

Anderson M.H., Molvar M.P., Powel L.V.: Treating dental caries as an infectious disease. Oper Dent, 1991, 16: 21-28.

Bjorndal L. Dentin and pulp reactions to caries and operative treatment: biological variables affecting treatment outcome. Endodontic Topics 2002, (1601-1538),10-23.

Bjorndal L. Dentin caries: Progression and clinical management. Operative Dentistry 2002: 27: 211-217.

Bjorndal L, Thylstrup A. A structural analysis of approximal enamel caries lesions on subjacent dentin reactions. Eur J Oral Sci, 1995, 103: 25-31.

Bjorndal L. Indirect pulp therapy and steways excavation. J Endod 2008, 34: 29-33.

Casagrande L, Bento LW, Dalpian DM, Garcia-Godoy. Direct pulp treatment in primary teeth: 4-year results. Am J Dent. 2010: 23:34-38.

Senawongse P, Otsuki M, Tagami M, Mjör IA. Morphological characterization of attrited human dentine. Arch Oral Biol 2008; 53:14-19

Briso ALF, Rahal V, Mestrener SR, Dezan E. Biological response of pulps submitted to different capping materials. Brazilian Oral Research, 2006, 20 (3), 219-225.

Espelid I., Fjeltveit K. Clinical diagnosis of occlusal dentin carries . Caries Res 1999; 28: 368-372;

Stevenson A.G., Beeley J.A.: The specificity of caries detector in cavity preparation. Br. Dent. J 2002; 176:417-421;

Pashley D.H.: Clinical correlations of dentine structure and function. J. Prosthet. Dent. 1991, 66: 777-781.

Al-Hiyasat AS, Barrieshi-Nusair KM, Al-Omari MA. The radiographic outcomes of direct pulp capping procedures performed by dental students. J Am Dent Assoc, 2006, 137: 1699-1705.
Costa CA, Giro EM, Nascimento AB, Teixeira HM, Hebling J. Short term evaluation of the pulpo-dentin complex response to modified glass-ionomer cement and a bonding agent applied in deep cavities. Dent Mater, 2003, 19: 739-746.
Snuggs H.M., Cox C.F., Powell C.S., White K.C.: Pulpal healing and dentinal bridge formation in an acid enviroment. Quintessence Int. 1993, 24: 501-510.
Faraco IM, Holland R. Response of the pulp of dogs to capping with MTA or calcium hydroxide cement. Dent Traumatol, 2001, 17 163-166.
Estrella CR, Bammann LL, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J. 2000: 11:3-9.
Reeves R, Stanley HR. The relationship of bacterial penetration and pulpal pathosis in carious teeth. Oral Surg Oral Med Oral Pathol, 1966: 22: 59-65.
Leye Benoist F, Gaye Ndiaye F, Kane AW, Benoist HM, Farge P. evaluation of Minerale Trioxid Aggregate versus calcium hydroxide cement (Dycal) in the formation of dentin bridge: a randomized controlled trial. Int Dent J, 2012, 62: 33-39.
Ferracane JL, Cooper PR, Smith AJ. Can interraction of materials with dentin-pilp complex contribute to dentin regeneration. Odontology, 2010, 98:2-14.
Accorinte MLR, Loguercio AD, Reis A, Carneiro E, Grande RHM, Murata SS, Holland R. Response of human dental pulp capped with MTA and calcium hydroxide powder. Operative Dentistry 2008, 33-5, 488-495.
Olsson H, petersson K, Rohlin M. Formation of a hard tissue barrier after pulp cappings in humans. A systematic review. Int Endod J, 2006, 39 (6), 429-442.
Olsson H, Petersson K, Rohlin M. Formation of a hard tissue barrier after pulp cappings in humans. A systematic review. Int Endod J, 2006, 39(6): 429-442.
Furey A, Hjelmhaug J, Lobner D. Toxicity of Flow line, Durafill, vs Dycal to dental pulp cells. Effects of growth factors. J Endod, 2010, 36: 1149-1153.