PULPAL RESPONSES AFTER CARIES TREATMENT IN HUMAN TEETH: AN IMMUNOHISTOCHEMICAL STUDY

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Abstract

The class II major histocompatibility complex (MHC) molecule expressing cells, termed dendritic cells and lymphocytes in human dental pulp are highly sensitive to exogenous antigenic stimuli. Their drastic changes in number and localization are induced by dental caries. This study investigated the responses of the immune system in 3 different clinical conditions: shallow and deep cavities and treated caries. Cells were identified immunohistochemically by using the following monoclonal antibodies: HLA-DR, CD45RO and CD20. Initial pulpal response was characterized by a localized accumulation of HLA-DR antibody-positive cells in the pulp tissue beneath the dentinal tubules communicating with the caries lesion. In the pulp of progressed caries, a large number of HLA-DR-positive cells was observed with a marked increase of other kinds of immunocompetent cells. This might indicate the occurrence of antigen presentation locally in the pulp tissue, which is very important for the immune response. In treated carious teeth, clusters consisting of HLA-DR-positive cells and CD45-positive T lymphocytes were recognized locally in the pulp tissue, regardless of cavity depth. CD20-positive B cells were seen only under the deeper cavities.

The results of this study demonstrated that dental pulps respond to cavity preparation and restoration, and that antigen presentation and cellular or humoral immunoresponses persist for many months, even after caries treatment.

Key words: human dental pulp, MHC class II molecule-expressing cells, lymphocytes, dental caries, caries treatment, adhesive system, immunohistochemistry

Introduction

Caries removal, cavity preparation, and restoration with adhesive systems are generally conducted in dental practices. There has been no shortage of papers published on the subject of histological evaluations of pulpal responses to cavity preparation in animals, but very little attempts to evaluate those in human teeth.

Dental pulp is equipped with major histocompatibility complex (MHC) class II molecule-expressing cells for initiating immune responses to exogenous antigenic stimuli. In intact teeth, they are distributed mainly in and around the layer of odontoblasts and are called pulpal dendritic cells. Drastic changes in their localization are induced by human dental caries [1, 2, 3, 4, 5], and after cavity preparation in rats [6, 7, 8, 9]. Analysis of these data suggests that class II molecule-expressing cells are highly sensitive to antigenic stimuli penetrating dentinal tubules [10, 11].

Caries attack also induces changes in the distribution of lymphocytes; they become concentrated beneath the carious lesions [12, 13]. Following the exogenous invasion of microorganisms, host defence reactions, such as inflammatory and immunological reactions, take place in the pulp in order to eliminate the foreign pathogens and to maintaince the local homeostasis in the pulp. Interactions between lymphocytes and MHC class II molecule-expressing cells have been shown in pulpal inflammation [14].

The focus of this paper is the influence of an operative procedure upon the distribution of MHC class II molecule-expressing cells and lymphocytes. We have investigated pulpal responses in untreated carious teeth compared with carious lesions treated with an adhesive system. We postulated that pulpal responses for cavities with caries and that with treated carious teeth would no longer have their responses after 6 months.

Material and method

We have examined 30 human teeth from patients at the age of 9 to 14 years. Teeth were extracted from various therapeutic reasons (mostly from orthodontic reason), and immediately cut longitudinally; pulp tissue was extirpated and fixed in formalin for 24 hours at 4°C. The specimens were embedded in paraffin, according to standardized laboratory procedure. Sections were cut at 5 ìm thickness and stained by the streptavidin-biotin complex immunoperoxidase method. Cells were identified immunohistochemically by using the following monoclonal antibodies: HLA-DR (for dendritic cells), CD45RO (for memory T - lymphocytes) and CD20 (for B -lymphocytes).

To verify our hypothesis, we analyzed pulpal responses in 3 different clinical conditions: shallow (n=10, pulp with caries in dentin, about 2-3 mm from the pulp chamber), deep cavities (n=10, pulp with caries deep into the dentin, 0.5-1.5 mm from the pulp chamber) and treated caries (n=10). Treatment of caries lesions was carried out on occlusal surfaces. The distance between cavity floors and pulpal walls varied from 0.5 to 3 mm. The Uni Fil Bond

dental adhesive system and GC Gradia composite resin were applied to the prepared teeth.

The depth of the carious lesion was determined by the pigmentation of hard tissues.

The main numbers of dendritic cells, T-cells and B-cells in each group were statistically analysed with ANOVA.

Results

The number of antigen-presenting and immunocompetent cells in each group is shown in Table 1 and Fig.1.

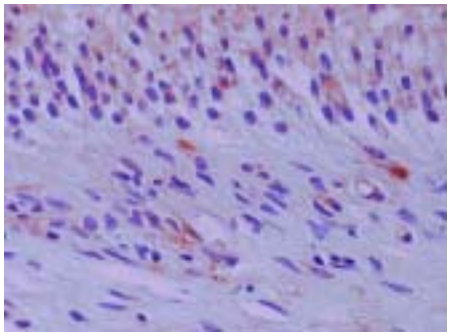

Fig. 1. Mean number of HLA-DR, CD45RO and CD 20 antibody positive cells in human dental pulp in shallow and deep cavities and treated caries

	Group 1	Group 2	Group 3
N	10	10	10
HLA-DR	5.0	22.1	2.2
CD45RO	19.5	89.6	7.1
CD20	4.7	51.2	1.5

Values are means ± SEM; N, number of samples

In shallow dentinal lesions a few HLA-DR cells were present and they were distributed mainly around an odontoblast layer and along the dentin-pulp border (Fig. 2).

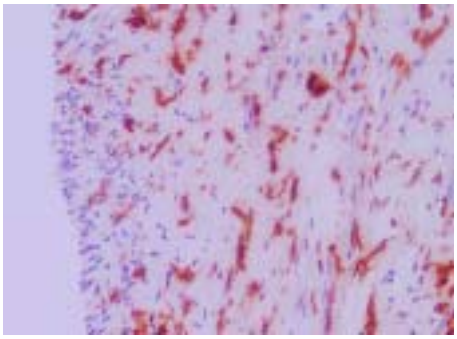

Fig. 2. Immunohistochemical localization of HLA-DR positive cells in the pulp with shallow cavities

As the caries lesion advanced, cells expanded toward the center of the pulp. Under deeper cavities HLA-DR-positive cells were dispersed among affected odontoblasts and they have displaced odontoblasts below the cavities. Cells were markedly increased, but not significantly (Fig.3).

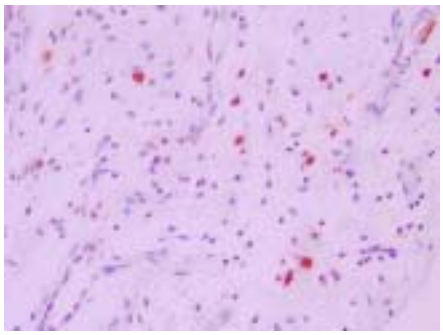

Fig. 3. Immunohistochemical localization of HLA-DR positive cells in the pulp with deep cavities

An increase of CD45RO-positive cells T-lymphocytes was observed in majority of specimens in teeth with moderate to deep caries. These cells were concentrated below the para-odontoblastic region, forming an aggregation. The number of T-cells was markedly increased in deep cavities and significant differences were evident between group 1 and 2 ($p<0.01$), (Fig. 4, Fig. 5).

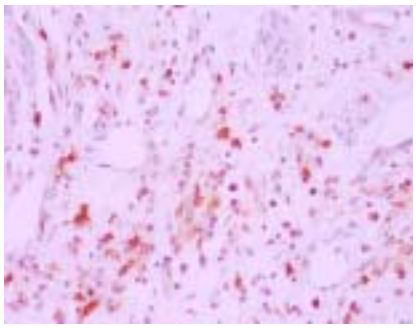

Fig. 4. Immunohistochemical localization of CD45RO positive cells in the pulp with shallow cavities

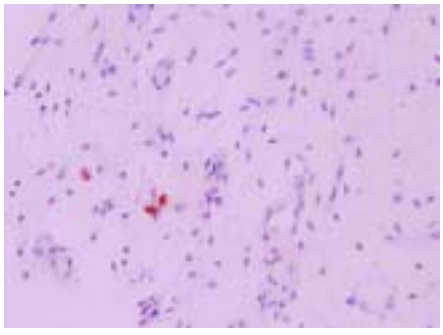

Fig. 5. Immunohistochemical localization of CD45RO positive cells in the pulp with deep cavities

The number of CD20-positive B-lymphocytes was much smaller than that of T-lymphocytes in most specimens. A considerable number of CD20-positive cells was detected among lymphocytes forming clusters in deeper cavities, with significant differences between group 1 and 2 ($p<0.01$), (Fig. 6, Fig. 7).

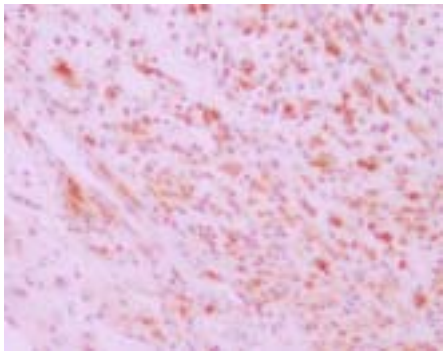

Fig. 6. Immunohistochemical localization of CD20 positive cells in the pulp with shallow cavities

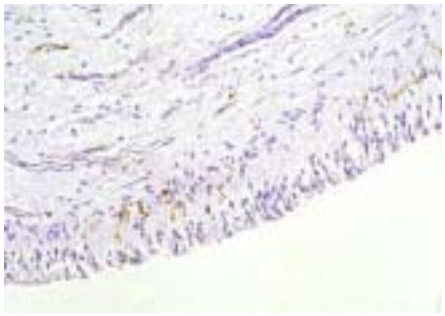

Fig. 7. Immunohistochemical localization of CD20 positive cells in the pulp with deep cavities

Treatment of caries lesions showed that beneath 9 of the 10 cavities samples, aggregations of HLA-DR-positive cells were recognized locally (Fig. 8), and always followed the accumulation of CD45RO-positive cells T-lymphocytes (Fig. 9). CD20-positive B-cells were seen only under deeper cavities (Fig.10). There were significant differences in the number of T-cells between group 3 and 1 ($p<0.05$), and between group 3 and 2 ($p<0.01$), and in the number of B cells between group 3 and 2 ($p<0.01$).

Discussion

This study provides evidence that cavity depth influences the distribution of HLA-DR-positive dendritic cells and lymphocytes. Reduction in the thickness of residual dentin had an impact on the distribution of the cells. These changes are in agreement with findings from studies on the distribution of dendritic cells and lymphocytes in human teeth. Early pulpal response to bacterial diffusion of bacterial products through dentinal tubules elicits the influx of dendritic cells, T-lymphocytes

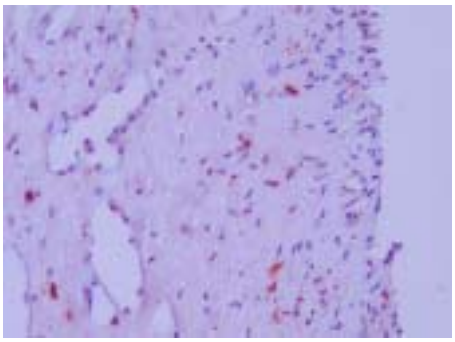

Fig. 8. Immunohistochemical localization of HLA-DR positive cells in the pulp of treated caries

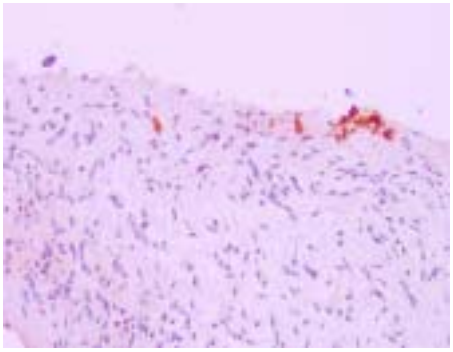

Fig. 9. Immunohistochemical localization of CD45RO positive cells in the pulp of treated caries

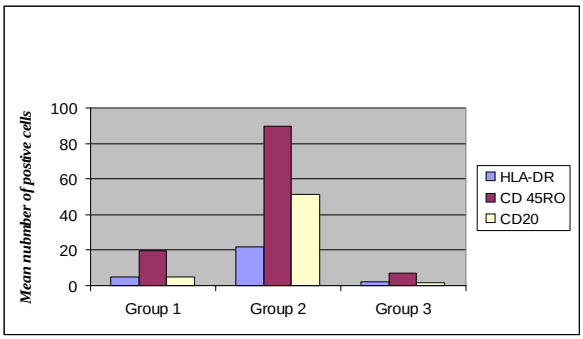

Fig. 10. Immunohistochemical localization of CD20 positive cells in the pulp of treated caries

and rare B-lymphocytes. As the infection is coming closer to the pulp, the response assumes a typical mixed character, consisting of T-cells and B-cells.

On the other hand, most components of adhesive systems and composite resins are able to diffuse through the dentinal tubules and reach the pulp tissue producing noxious effects on odotoblasts (15,16) and influence the function of pulpal immunocompetent cells [17, 18, 19, 20]. In meanwhile, no aggregations of dendritic cells were recognized under prepared cavities, and no aggregations of CD45-positive lymphocytes were detected in any sample of the cavity prepared teeth. Thus, the materials

used here provided excellent sealing characteristics, and they effectively prevented the ingress of noxious substances to the dentin-pulp complex.

Decrease in the number of dendritic cells and lymphocytes were recognized under prepared cavities, with a statistical significance between caries-affected teeth and after treatment. Against our expectation, even after caries treatment, small aggregations of HLA-DR-positive dendritic cells, accompanied by CD45-positive cells T-lymphocytes, were left behind in 9 out of 10 samples. The inflamed lesions would be the result of activities by bacteria, which had existed locally deep in the dentinal tubules and survived even after the removal of caries. Presence of dendritic cells and lymphocytes after removal of the carious lesion shows that local antigen presentation and cellular and/or humoral immunoresponses persist even after careful treatment of caries.

Conclusion

Our study demonstrated that dental pulps have different response to cavity preparation and restoration, and that antigen presentation and immunoresponses persist for many months, even after caries treatment. Further investigations are needed to ascertain how to control the bacterial activities that might have remained deep in the dentinal tubules.

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VASCULAR PATHWAYS OF HUMAN DECIDUOUS DENTAL PULP

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Abstract

The transition from deciduous to permanent teeth is a unique and dynamic process in which the development and eruption of permanent teeth is coordinated with the resorption of deciduous teeth.

The vascularisation in pulp tissue of human deciduous teeth has not been as well studied as that within human permanent teeth. Such information is useful to those who diagnose and treat children's teeth.

In order to examine the reparatory pulp ability of deciduous teeth, it was our aim to determine vascular structures in contrast to the histological appearance of noncarious human primary teeth with root completion and physiological resorption, because the dental pulp is an active component in the life of the tooth.

Histological examinations of blood vessels in deciduous dental pulp were performed on a light microscope and on a Transmission Electron Microscope.

When the process of physiological root resorption in deciduous teeth is initiated, blood vessels in pulp tissue show some disturbances. Another area in which information is lacking involves changes in the blood vessels of the deciduous teeth during the period of root resoption. Endothelial cells of arterioles, venules and capillaries are cuboidal, with large pinocytotic vesicles, progressive reduction of luminal capacity, and reduction of the wall of the pulp blood vessels.

Key words: deciduous teeth, dental pulp, root resorption, blood vessels, ultrastructure

Introduction

The transition from deciduous to permanent teeth is a unique and dynamic process in which the development and eruption of permanent teeth is coordinated with the resorption of deciduous teeth. Primary teeth contrary to permanent ones, have a relatively short lifetime and functional duration, and are subordinated to an early physiological resorption of the roots.

In the past human primary teeth has received little attention compared to similar research on permanent teeth, because they are smaller and short lived, and due to the belief that the pulps are similar. Now a days, deciduous dental pulp is the origin of stem cells and is progenitor for tissues with therapeutic expectations in much disease in future[1, 4].

Dental pulp is a unique tissue, responsible for the tooth vitality, and when this tissue is damaged by disease; it reacts in an attempt to defend by production of protective dentine. From its non-specific and specific defensive mechanisms, depends the survival of the tissue in pathological conditions. This tissue passes three phases: phase of root formation, phase of functional duration and phase of physiological resorption of the root.

The vascularisation in pulp tissue of human deciduous teeth has not been as well studied as that within human permanent teeth. Such information is useful to those who diagnose and treat children's teeth.

Another area in which information is lacking involves changes in the blood vessels of the deciduous teeth during the period of root resorption.

Aim: In order to examine the reparatory pulp ability of deciduous teeth, it was our aim to determine vascular structures in contrast to the histological appearance of noncarious human primary teeth with root completion and physiological resorption, because the dental pulp is an active component in the life of the tooth.

Methods

The pulps used for this research had originated from intact teeth of healthy children, aged 5 to 9 years (5 deciduous teeth without signs of physiological resorption, and 5 deciduous teeth with progressive physiological resorption).

Immediately after the extraction (performed due to orthodontic reasons, under local anesthesia), each tooth was cut perpendicularly to its long axis with a rotating carborundum disc under a water jet. The separated halves were dissected with plastic instrument, and the tooth pulp was excavated completely.

The histological examinations were performed on a light microscope Orthoplan Leitz-Wetzlar with haematoxyllin-eosin stain (HE), and on a Transmission Electron Microscope (TEM) Tesla BS 500 (60KV).

Results

The vascular structures of deciduous dental pulp were studied in human deciduous teeth using a light microscope and a TEM (Fig. 1, 2, 3). Their fine structure corresponds with blood vessels in other tissues, and the architectural morphology is similar to that of permanent pulps, according to Rapp[5].

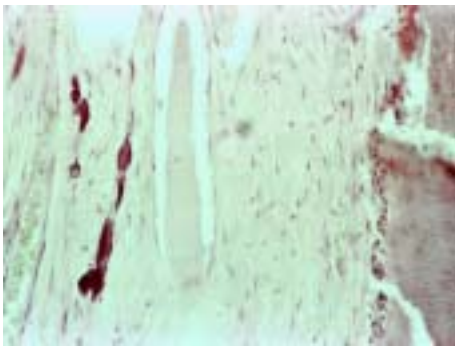

Fig. 1. Deciduous teeth without progressive physiological resorption: dentin and pulp tissue with arterioles, venules and capillaries with endothelial cells and pericytes, with red blood cells, and calcifications (HE, 400x)

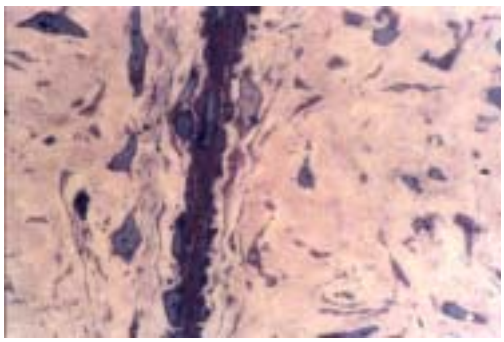

Fig. 2. Deciduous teeth without progressive physiological resorption: arterioles in pulp tissue with endothelial cells and pericytes (HE, 1000x)

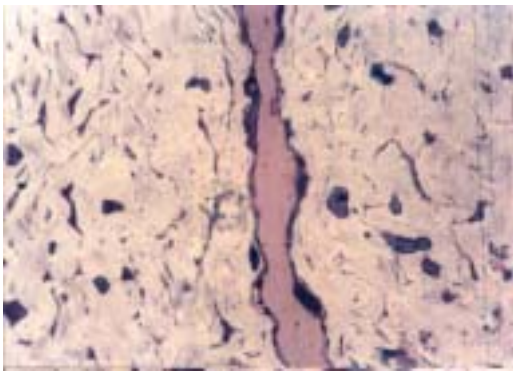

Fig. 3. Deciduous teeth without progressive physiological resorption: venula in pulp tissue (HE, 1000x)

Endothelial cells are flat cells with an ovoid nucleus in the middle of the cell, which often give the luminal side of the cell a bulging contour, mostly prominent in capillaries.

Vascularisation of deciduous dental pulp in the second group of teeth, when the physiological resorption had been started, showed some disturbances. Endothelial cells were cuboidal, with large pinocytotic vesicles, like an atheromathosis with progressive reduction of luminal capacity, and reduction of the wall of the pulp blood vessels (Fig. 4, 5, 6, 7). In addition, development of edematous changes of the basic substance with perivascular hyalinization was observed (Fig. 6). It spoke in favor of progressive disturbance of nutrition, resulting in reduction of cells and collagen net.

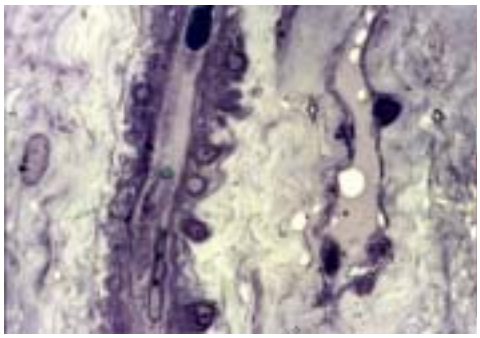

Fig. 4. Deciduous teeth with progressive physiological resorption: cuboidal endothelial cells with large pinocytotic vesicles (lipid material), perivascular hyalinization (HE, 1000x)

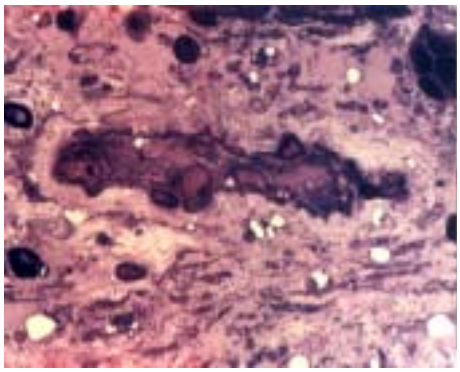

Fig. 5. Deciduous teeth with progressive physiological resorption: cuboidal endothelial cells (HE, 1000x)

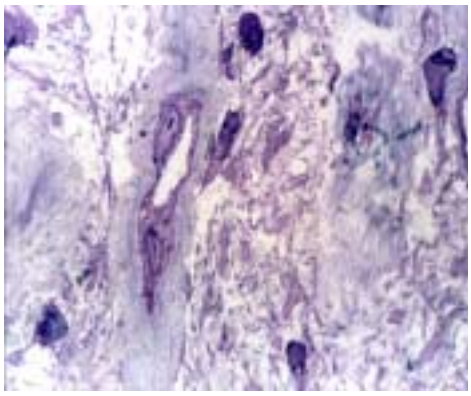

Fig. 6. Deciduous teeth with progressive physiological resorption: cuboidal endothelial cells, perivascular hyalinization (HE, 1000x)

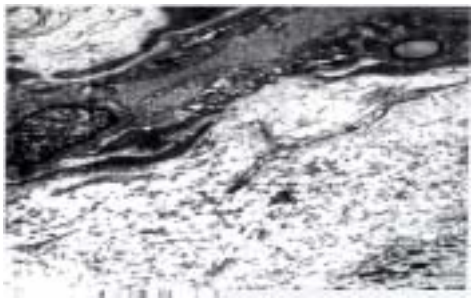

Fig. 7. Deciduous teeth with progressive physiological resorption: cuboidal endothelial cells with large pinocytotic vesicles (lipid material) (TEM, 17000x)

Discussion

Physiological resorption of the root in deciduous teeth is a complex process, and unique in human organism: there is no other organ that is mineralized that after finishing its function it demineralizes and sheds. However, the increasing need of child's nutrition for growth depends on this.

When the process of physiological root resorption in deciduous teeth is initiated, the dental pulp enters in phase of involution, tending to decrease blood supply and innervations, accordingly the results of Yu & Abbott[2]. Blood vessels of the pulp show signs of stasis and perivascular calcifications. Under the light microscopy, regressive changes are evident in the blood vessels, as a result of edematous changes in the basic substance and perivascular hyalinization (Fig. 4, 5, 6, 7).

In the cytoplasm of the endothelial cells under TEM, there are to many pinocytotic vacuoles incorporated inside the cells, full of lipid material, because of their strong micropinocytotic activity - ingestion of saturated fats.

Because of the aforementioned changes in the endothelial cells, pulp blood vessels supply is compromised. These unavoidable circumstances lead to a decrease in all pulp functions.

With age, the pulp tissue reduces in size[3]. In our opinion, physiological resorption of the root produces consequences to deciduous dental pulp similar to aging - with age, nerve and blood supply to the pulp tend to decrease, and the pulp becomes more fibrous and less cellular (Yu & Abbott[2]).

Conclusions

Physiological resorption of the root produces consequences to deciduous dental pulp similar to aging - with age, nerve and blood supply to the pulp tends to decrease.

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PREVENTION OF WHITE SPOT LESIONS DURING ORTHODONTIC TREATMENT

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Abstract

The purpose of this *in vitro* study was to evaluate the effect of a paste containing CPP-ACP, GC Tooth Mousse in preventing white spot lesions (WSL) during orthodontic treatment with fixed appliances.

A total of 30 extracted human lower and upper premolars with no restorations, cracks, caries, hypoplastic areas or pliers impressions were collected for this study and used within one and six months. Enamel of buccal surface of the teeth was polished with pumice and water, rinsed and air dried. After etching the enamel surface with a 37% phosphoric acid solution for 15 seconds and rinsing for 10 seconds, teeth were dried. In this study *Ricketts Universal Ultratrim* (*Dentaurum, Germany)* stainless steal brackets for premolars were used. Each bracket was positioned over the mid point of the clinical crown on buccal surfaces of the prepared premolar. Con Tec LC (*Dentaurum, Germany*) was used as adhesives for bonding brackets in this study. The teeth were divided randomly into three groups, each one consisting of 10 teeth. The first group - control group included samples without any prevention; the second group included samples which enamel surface was treated with dental cream (GC Tooth Mousse) each day for 5 minutes in a period of 1 month and the third group included samples which enamel surface was treated with dental cream (GC Tooth Mousse) each day for 5 minutes in a period of 3 months.

Teeth were stored in artificial saliva for one or 3 months. After that, the samples were prepared for SEM analysis (JEOL JSM 5300), using sputter technique in a vacuum evaporator. The appropriate area of enamel surface was analyzed in order to determine micro morphology changes in the structure of the enamel, on the place of previous brackets fixation.

The application of GC Tooth Mousse dental cream after bonding appears to be beneficial in reducing the incidence of white spot lesions.

Key words: white spot lesions, brackets, prevention

Introduction

Enamel demineralization or white spot lesions around orthodontic fixed appliances is a common side effect of orthodontic treatment [1]. These orthodontic appliances tend to cause a shift of the lesion from posterior to anterior teeth and from interproximal to vestibular and lingual sites. Considering the mechanical difficulties of removing plaque with orthodontic brackets in place, proper oral hygiene is crucial. Unfortunately, patient compliance is a commodity that is unpredictable and decreasing. Consequently, the incidence of enamel decalcification and caries during orthodontic care is increasing [2]. For example, Gorelick et al. [3] found white spot lesions for nearly 50% of patients that underwent orthodontic treatment. In addition, Øgaard et al. [4] reported that these lesions can develop within 4 weeks or the average time between orthodontic visits. For a specialty whose objectives are to improve facial and dental esthetics, the presence of unsightly white spot lesions may detract from the beneficial effects of orthodontic treatment.

Many products have been developed to prevent demineralization of enamel surface. One such product is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). CPP-ACP can be found in multiple products [5, 6].

Recaldent™ is a unique complex containing amorphous calcium phosphate (ACP) and casein phosphopeptide (CPP), obtained from milk casein. The preparation is recommended in hard tissue remineralization. The manufacturer compares the material to "liquid enamel". CPP-ACP complex makes a strong binding with dental biofilm and form calcium and phosphate reservoir. They are then incorporated into the surface of enamel and dentine [7]. The CPP-ACP complex contained in Recaldent™ is hence an ideal system for transporting free calcium and phosphate ions - and GC

Tooth Mousse is the world's first product for professional use in the dental practice that contains this novel active ingredient [8]. The proposed anticariogenic mechanism of CPP-ACP involves the incorporation of the nanocomolexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir. Studies have shown that CPP-ACP incorporated into dental plaque can significantly increase the levels of plaque calcium and phosphate ions. This mechanism is ideal for the prevention of enamel demineralization as there appears to be an inverse association between plaque calcium and phosphate levels and measured caries experience [9].

Some of authors [10, 11] who had evaluated the incidence of carious process influenced by input of CPP-ACP compound, demonstrated that this compound reduced the incidence of carious lesions when entered in the form of chewing gums. The chewing gums are ideal for transport of CPP-ACP compound in the mouth, because in such way it remains in the mouth long enough and shows its beneficial effect. Its presence in the plaque is confirmed after three hours later the chewing gum [12, 13].

The purpose of this *in vitro* study was to evaluate the effect of a paste containing CPP-ACP, GC Tooth Mousse in preventing white spot lesions (WSL) during orthodontic treatment with fixed appliances.

Material and methods

A total of 30 extracted human lower and upper premolars with no restorations, cracks, caries, hypoplastic areas or pliers impressions were collected for this study and used within one and six months. All extractions were indicated for orthodontic purposes in patients of 11-18 years of age. After being extracted, teeth were carefully inspected and only intact teeth were cleaned and stored in artificial saliva. The artificial saliva contained KCl (1.04 g/L), NaH_2PO_4 (0.68 g/L), $NaHCO_3$ (0.42 g/L), $CaCl_2$ (0.03 g/L) and $MgCl_2$ (0.01 g/L).

Enamel of buccal surface of the teeth was polished with pumice and water, rinsed and air dried. After etching the enamel surface with a 37% phosphoric acid solution for 15 seconds and rinsing for 10 seconds, teeth were dried. In this study *Ricketts Universal Ultratrim* (*Dentaurum, Germany)* stainless steal brackets for premolars were used. Each bracket was positioned over the mid point of the clinical crown on buccal surfaces of the prepared premolar. Con Tec LC (Dentaurum, Germany) was used as adhesives for bonding brackets in this study. The teeth were divided randomly into three groups, each one consisting of 10 samples:

- first group control group: without any prevention;

- second group: samples which enamel surface was treated with dental cream (GC Tooth Mousse) each day for 5 minutes in a period of 1 month;

- third group: samples which enamel surface was treated with dental cream (GC Tooth Mousse) each day for 5 minutes in a period of 3 months.

Teeth were stored in artificial saliva for one or 3 months. After that, the samples were prepared for SEM analysis (JEOL JSM 5300), using sputter technique in a vacuum evaporator. The appropriate area of enamel surface was analyzed in order to determine micro morphology changes in the structure of the enamel, on the place of previous brackets fixation.

Results

The analysis of enamel surface was performed using a scanning electronic microscopy after completion of a particular preventive treatment and bracket debonding. Comparison was made between the buccal surface of the tooth on which the brackets were fixed, and those specimens with no to prevention. In this group, micromorphology characteristics showed decomposed enamel surface with lost integrity (Fig. 1).

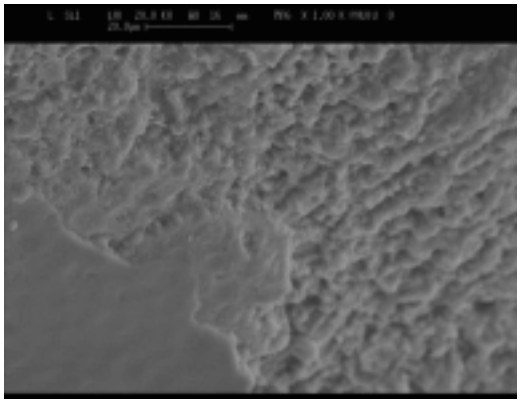

Fig. 1. SEM images of tooth samples from the control group: lost enamel integrity; enamel decomposition (original magnification, x 1000)

In the second group, comprising tooth samples that were prevented with dental cream (GC Tooth Mousse) each day for 5 minutes in a period of 1 month, inhibited demineralization of enamel in the center and on the outskirts of enamel prisms was noted (Fig. 2).

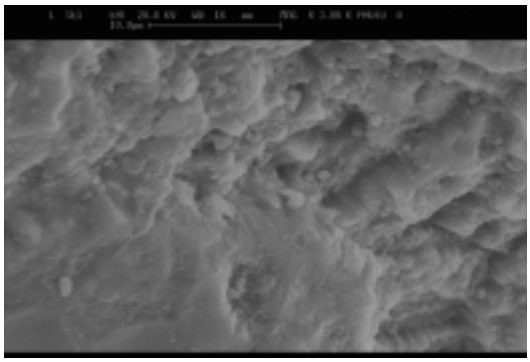

Fig. 2. SEM images of tooth samples from the second group: inhibited demineralization of enamel in the center and on the outskirts of enamel prisms (original magnification, x 1000)

In the third group comprising tooth samples that were prevented with GC Tooth Mousse each day for 5 minutes in a period of 3 months, presence of amorphous deposits on the enamel surface was observed (Fig. 3).

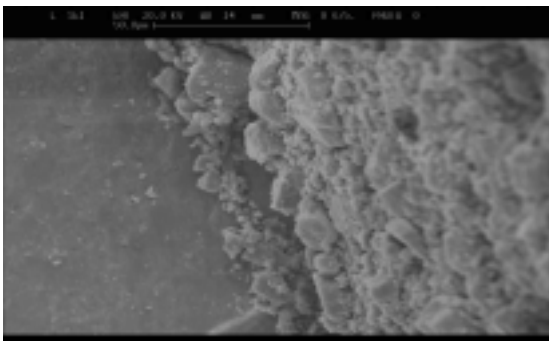

Fig. 3. SEM images of tooth samples treated with GC Tooth Mousse; presence of amorphous deposits on the enamel surface

Discussion

The most common negative effect of orthodontic treatment with fixed appliances is the development of incipient carious lesions around brackets and bands, particularly in cases with poor oral hygiene. Caries lesions typically form around the brackets interface, usually near the gingival margin (Fig. 4 a, b). Since orthodontic appliances make plaque removal more difficult, patients are more susceptible to carious lesions. Mineral loss (demineralization) or gain (remineralization) by enamel is a dynamic physicochemical process occurring when oral bacteria form a biofilm on the enamel surface and this biofilm is exposed to fermentable dietary carbohydrates, sucrose being the most cariogenic of them [14]. Thus, every time sugar penetrates into a cariogenic biofilm and is converted to acids by bacterial metabolism, the biofilm fluid becomes undersaturated with respect to the enamel mineral, and demineralization occurs [15].

Administration of topical agents containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), maintenance of oral hygiene, and dietary control have been suggested as mechanisms to control the formation of enamel lesions during fixed orthodontic appliance treatment [16]. In contemporary orthodontic literature, fluoride and CPP-ACP applications are accepted approaches for remineralizing of the previously demineralized enamel. CPP-ACP is known to be a source of calcium and phosphate close to the sites of possible demineralization, and this is likely to inhibit demineralization, enhance remineralization or possibly both [17]. In our study the tooth treated with CPP-ACP was remineralized by calcium and phosphorus, and the resulting calcium-phosphate layer was found to be amorphous. Previous studies have demonstrated that CPP-ACP enhances the remineralization of artificially formed

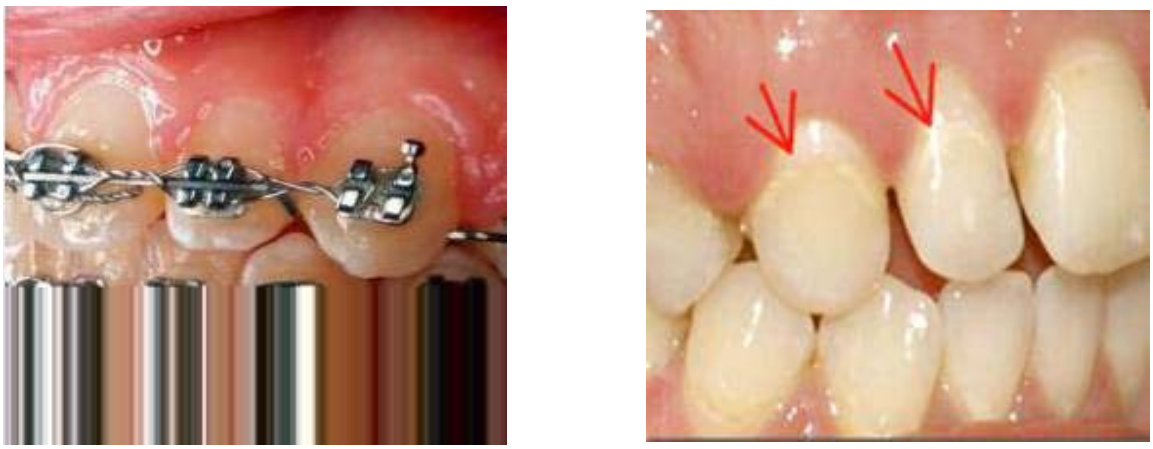

Fig. 4 (a, b). Incipient caries lesions (white spot) develop around brackets and bands due to poor oral hygiene

dentinal lesions. The suggested mechanism for this is the stabilization of calcium phosphates on the tooth surface by the casein phosphopeptides, which leads to high concentration gradients of calcium and phosphate ions, thus promoting the remineralization of hard tissues [18].

The role of CPP-ACP has been described as localization of ACP on the tooth surface, which buffers the free calcium and phosphate ions. This helps to maintain a state of supersaturation with respect to the enamel by suppressing demineralization and enhancing remineralization [19]. Enamel lesions, which were remineralized with topical exposure to CPP-ACP, have been shown to be more resistant to subsequent acid challenge compared with normal remineralized enamel as CPP-ACP is able to promote the remineralization of enamel subsurface lesions with hydroxyapatite. In addition, the relativity low carbonate environment of the CPP-ACP treated subsurface lesion may also exhibit both improved crystallinity and lower microstrain than might be found in normal tooth enamel [20].

Conclusions

Within the limitations of an *in vitro* study, the results lead to conclusion that dental cream containing CPP-ACP enhances the remineralization potential of the enamel in teeth.

The application of GC Tooth Mousse dental cream after bonding appears to be beneficial in reducing the incidence of white spot lesions.

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DETERMINING CORONARY MICROLEAKAGE OF ENDODONTICALLY TREATED TEETH RESTORED WITH TEMPORARY AND PERMANENT RESTORATIONS

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Abstract

The aim of the study was to determine the coronary microleakage in in-vitro conditions of the endodontically treated teeth, restored with temporary and permanent fillings.

Material: The experimental part of the study included a total of 60 intact single root human teeth, which were endodontically treated in maximum sterile conditions. The intracanal obturation was made with the sealer AH plus and Termafill gutapercha technique. The teeth were divided in 3 groups depending on the coronary restoration: first group - composite resin, second group - Caviton and third group - dental amalgam. Next, we sterilized the teeth with water steam at temperature of 121°Ñ and pressure of 1.5 in autoclave in duration of 15 minutes. After isolating the surface of the roots with two layers of nail polish to the enamel-dentin border, the teeth with the crowns were submerged into bacterial suspension prepared from Proteus mirabilis, Gram- negative motile, rod shaped bacteria, with concentration of the bacterial cells of 10^7 - 10^9 in milliliter of solution. They stayed in the bacterial suspension at temperature of 37°Ñ and permanent bacterial concentration for 5 to 30 days. After the time interval of 5 and 30 days, we prepared the teeth for the procedure of histological evaluation of the coronary microleakage and the longitudinal sections were colored by Brow-Bern.

Results: The largest bacterial microleakage after 5 days was determined in the second test group of the teeth restored with the temporary filling material - Caviton (80%). After the period of 30 days, the bacterial microleakage was largest in the second test group and it was 70%.

Conclusion: Coronary restoration as a final procedure in the endodontic therapy should be realized in a period of 5 days, so that the contamination of the endodontic space can be stopped.

Key words: microleakage, endodontic therapy, composite resin, dental amalgam

Introduction

The microleakage is defined as a physical movement of fluids and microorganisms from the oral cavity to the cavities through the endodontic space to the apical and periapical spaces. This discrete migration, which the endodontists studied in the sixties of the past century [1], was pointed out as a possible reason for breaking out the integrity of the coronary as well as of the intracanal filling. This can cause contamination, dissolving of the cement, creation an empty spaces and transport of infectious material out of the tooth root into the periapical zone [2, 3]. Subsequently, the success of the endodontic therapy is compromised, the patient feels discomfort; there is a need for retreatment or surgical intervention, and finally, the treated tooth is lost.

Many studies suggest that microleakage, no matter if it is coronary or apical, has effect on the success of the endodontic treatment [4, 5, 6, 7]. The researches are mostly conducted in in-vitro conditions by evaluation the microleakage of colored solutions, radioisotopes, bacterial markers etc. [1, 4, 5]. Some suggest that the apical microleakage has a primary role in the endodontic therapy failure but also the importance of the coronary microleakage can not be neglected or forgotten. After all, tridimensional hermetically intracanal obturation means sealing of the apical and the coronary part of the tooth and it is one of the conditions for successful and prognostic positive endodontic procedure [6, 7, 8].

According to Siquera et al. [9] the most intensive bacterial leakage begins from the 2 th to the 58th day, and the authors did not find any difference in the level of the salivary coronary microleakage. Although the contamination of the canal system in the coronary parts was evident, the authors thought that in-vivo conditions should be accepted with a certain reserve.

Trope, Chow and Nissan [10], alerted that endotoxin was present in the canal chamber after 24 hours, and that quality coronary restoration provided healthy periradical status.

Chailertvanitkul et al. [11] in their study of coronary microleakage, with the tracer bacteria *Fusobacterium nucleatum* and did not detect permeability

in the group where the opening was covered with modified glass ionomer cement after a period of 60 days.

The therapeutic endodontic procedure has to be realized in one session, which is not always possible, and the coronary accessory cavities are closed with temporary sealing materials [12, 13, 14]. These same materials, in the processes of mastication and loading are breaking, they fall out and the endodontic space is exposed to the conditions of the oral cavity and the possibility of contamination and infection [4, 7, 8, 15].

The contradictory literature findings about the role of the coronary microleakage in the failure of the endodontic therapy motivated us to set the aim of this research: to evaluate bacterial micropermeability of the endodontically treated teeth restored with temporary and permanent fillings.

Material and method

For realization of the aim of this research we used 60 single root intact human teeth, extracted for orthodontic reasons. They were cleaned with a scalpel from the tissue residues and until the moment of processing they were left in saline at temperature of 37°Ñ in order to remain wet and not modify the tissue structure. In maximum sterile conditions we did endodontic treatment of all teeth, irrigation with 2% solution of hypochlorite and canal obturation with sealer AH plus and Thermafil gutapercha technique. According to the coronary restoration, we divided the teeth in 3 groups:

- first group - 20 single root teeth endodontically treated and coronary restored with composite resin Tetric ceram and Excite adhesive system;

- second group - 20 single root teeth endodontically treated and coronary restored with temporary sealant Caviton;

- third group - 20 single root teeth restored with dental amalgam.

The teeth were left for 48 hours into saline at temperature of 37°Ñ, in order to organize and stabilize the filling. The next step was sterilizing of the tested specimens for 15 minutes, with water steam, in autoclave, at temperature of 121°Ñ and pressure of 1.5 at. The coating of the specimens root surface with two layers of nail polish to the enamel-cement border followed. Then we put the teeth in bacterial suspension made out of *Proteus mirabilis*, Gram-negative motile, rod-shaped bacteria, with concentration of the bacterial cells of 10^7 - 10^9 in milliliter of artificial saliva. Ten of the teeth stayed into the suspension for 5 days and the rest of them for 10-30 days, at temperature of 37°Ñ, and during that time, the bacterial concentration was closely monitored for an eventual change. Next, after removing the nail polish, the teeth were decalcified with Osteomol^R (Merck), for decalcifying of the hard teeth tissues, molded into paraffine blocks from which we made longitudinal sections with thickness of 5 ìm. We colored them by *Brow-Bern*, a coloring technique for histological evaluation of the bacterial presence. Verification of the microbiological microleakage of the crown to the apex of the teeth was made on bioocular microscope Eclipsa 600.

The final results were statistically analyzed with the computer program Statistica for Windows, version 6.

Results

Determination of bacterial coronary microleakage in a period of 5 days showed that out of total 10 teeth, bacterial migration was found in 30% of the composite group, 20% in the group with dental amalgam, and the most prominent bacterial permeability was found in the temporary restored teeth (in 80% of the specimens), (**Table 1**).

Table 1. Percentage representation of the bacterial microleakage after 5 days

leakage 5 days	**without**		**with**	
	no	%	no	%
l gr.	7	70.0	3	30.0
ll gr.	2	20.0	8	80.0
lll gr.	8	80.0	2	20.0

In the period of 30 days, changes in the bacterial flow from the coronary level were not observed in the groups restored with composite and dental amalgam. In the teeth closed with temporary sealing Caviton, a decrease of 10% was observed on the bacterial penetration compared with the period of 5 days (**Table 2**).

Table 2. Coronary bacterial microleakage after 30 days

leakage 30 days	**without**		**with**	
	no	%	no.	%
l gr.	7	70.0	3	30.0
ll gr.	3	30.0	7	70.0
lll gr.	8	80.0	2	20.0

Statistical analysis of the differences in the coronary bacterial microleakage according to the type of the restoration with the ANOVA test showed a statistically significant value of $p<0.05$. A significant difference was

Table 3. Coronary bacterial microleakage according to the type of restoration

leakage 5 days	Mann-Whitney U test rank sum gr 1	rank sum gr 2	U	Z	p	*sig. / n.sig.*
l gr / lll gr	200.0	110.00	45.00	(-) 0.38	0.7n.	sig.
ll gr / lll gr	75.00	135.00	20.00	(-) 2.77	0.02	sig.„
l gr / ll gr	80.00	130.00	25.00	(-) 2,19	0.03	sig. „

found between the temporary and permanent restorative materials on the coronary level; the difference between amalgam/Caviton was significant (p=0.02), as well as the difference between composite/Caviton (p=0.03). The samples of the second group showed much larger bacterial microleakage (**Table 3**).

The F-test analysis of variance (one-way ANOVA) used for testing the differences in the bacterial permeability after 30 days showed no statistically significant difference (p>0.05).

The Wilcoxon Matched test was used for evaluation of the bacterial microleakage from the coronary level in the groups for different time intervals (5 and 30 days). The difference between tested variables was insignificant (p>0.05), (**Table 4**).

Table 4. Bacterial microleakage for different time intervals

leakage termafil 5 / 30 days	Wilcoxon Matched test Z	p	*sig. / n.sig.*
l gr.	0.0	1.0	n.sig.
ll gr.	0.4	0.68	n.sig.
lll gr.	0.53	0.59	n.sig.

The following images are from the longitudinal histological sections with and without bacterial microleakage.

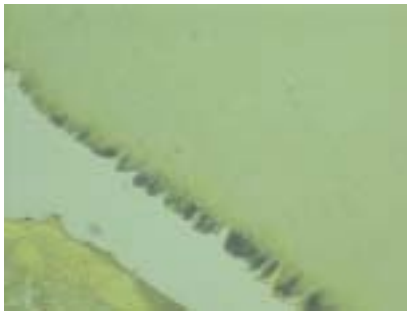

Fig. 1. Microleakage in group 1, after 5 days

Fig. 2a. Bacterial penetration in group 2, after 5 days

Fig. 2b. Bacterial penetration in group 2, after 5 days

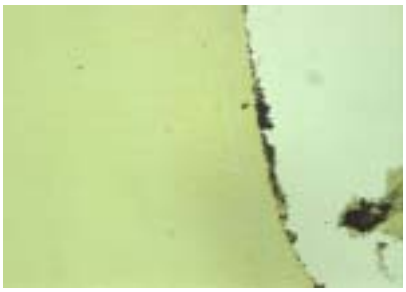

Fig. 3. Leakage in group 3, after 5 days

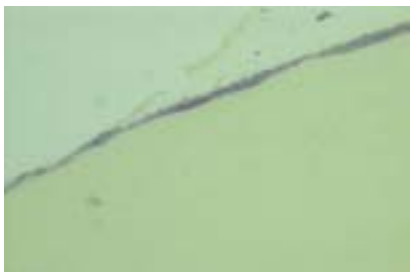

Fig. 4. Bacterial leakage after 30 days - group 1

Fig. 5a. Bacterial leakage after 30 days - group 2

Fig. 5b. Bacterial leakage after 30 days - group 2

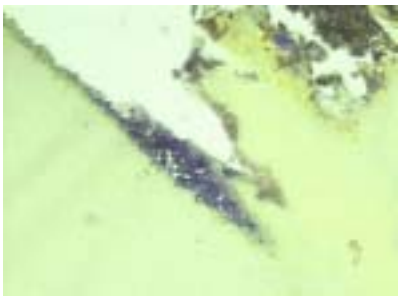

Fig. 6. Bacterial leakage after 30 days - group 3

Discussion

Microleakage, independent from the level of the crown or in the apical part, was the primary problem which endodontists had faced in their clinical practice [2, 3, 4, 5, 6, 7]. This factor, which prospectively compromises the success and the prognostic outcome of the endodontic therapy, has initiated the scientists to promote different methodologies which will, more or less, successfully determine, follow and prevent this process [1 ,5, 7, 11].

Microleakage is not just a transport of fluids, but also a movement of microorganisms and their metabolic products [9, 10, 11, 16, 17]. In order to get a real picture of the coronary leakage and the possibility for penetrating into the intra-canal space we determined the flow of the bacteria *Proteus mirabilis*, classfied as the most motile bacteria.

We determined different values of the coronary microleakage. The largest presence of *Proteus mirabilis* in the period of 5 days was registered in the second group where the specimens were coronary sealed with Caviton. The presented differences in the bacterial microleakage between the groups showed a statistically significant difference ($p<0.05$) as a result of the significantly higher permeability for bacteria of the temporary material for the coronary obturation in the second tested group.

Mann-Withney U test showed that the bacterial microleakage was not statistically significant between the permanent dental materials.

In the period of 30 days, there were no registered changes in the bacterial microleakage of the specimens in the first and the third group, while in the second group the values dropped for 10%, compared with the period of 5 days.

Statistical analysis of the differences in the coronary microleakage of *Proteus mirabilis* in the groups in the time interval of 30 days with the ANOVA test showed no significant difference, which was also confirmed with the Mann-Withney U test ($p>0.05$).

We evaluated the bacterial permeability from the crown to the endodontic space with the Gram-negative rod-shaped bacteria *Proteus mirabilis* with great potential for migration. The penetration of the bacteria was determined in two different periods (5 and 30 days) by a specific method of coloring and we verified the bacterial leakage in histological longitudinal sections. The permeability of Caviton for bacteria was confirmed in both tested periods in the second group. In the period of 5 days the observed microleakage was with greater intensity and resulted in higher significant difference. Similar data were also registered by Deveaux [16]. In his *in vitro* study he measured bacterial leakage of materials for temporary coronary obturation for 7 days and he suggested that the thickness of the material of 4 mm was optimal for reducing the microleakage independent of the thermocyclic procedure.

Contrary to our findings, Magura [18] on the longitudinal histological sections did not verify presence of bacteria with the coloring technique Brow-Brenn and did not find a statistically significant difference in the permeability of the temporary obturated and not sealed teeth. But, he highlighted that after 3 months the penetration of the artificial saliva statistically increased. Unlike his results, we determined coronary bacterial flow, using the same technique of coloring in the tested groups showing statistically significant values according to the material for coronary obturation and in both time intervals (period of 5 and period of 30 days).

In the period of 5 days the bacterial flow was minimal in the specimens of the first group where the occlusal cavities were obturated with composite resin. This result was in agreement with the results of Deveaux [16] and Sousa et al [19].

The dental adhesive along with the composite resin provided minimal bacterial microleakage in both tested periods. In our opinion, this is a result of its quality characteristics. Newer generations of dental adhesives like Excite, also have antimicrobial potential which allows reduction of the permeability of the bacteria. The correct layered application of the composite resin and the hybride texture in combination with the adhesive did not allow bacterial micro-flow in 70% of the tested teeth in both tested periods. These results coincide with the findings of Chailertvanitkul [11]. For preventing the coronary flow of oral fluids and microorganisms in the endodontic space they also suggested subjecting the orificium to dental adhesive.

The incidental bacterial microleakage of 30% in both first and third tested group suggests that the permanent restorative materials minimize the coronary leakage. On the other side, we confirmed that the definite coronary restoration of the endodontically treated teeth must be made in a period of 5 days if we want a successfull realization of the therapeutical procedure and positive outcome. Similar findings were also presented by •ivkoviæ [20] who in a period of 72 hours tested the apical bacterial permeability and found presence of bacteria in the dentine tubules independent of the used intra-canal cements.

In the period of 30 days microleakage in the second group was reduced due to cohesion and water expansion of the material for temporary sealing in the suspension. In clinical conditions, during the mastication processes, durability of these fillings is limited, or they break or fall out, and rarely keep their integrity [13, 15, 16, 18]. In *in-vitro* conditions we got these findings, but *in-vivo* researches are also needed for a complete clinical implementation.

We set a period of 5 and 30 days to expose endodontically treated teeth to bacterial suspension. The first tested date was imposed as a maximum time interval in which the endodontic procedure is desirable to be finished and with quality coronary resoration. The sealing of the transitory endodontic cavities is necessary to be realized in the shortest possible period of time after endodontic permanent canal obturation [8, 13, 21, 22, 23, 24].

Conclusion

The analysis and the evaluation of the results obtained have shown that the bacterial coronary microleakage exists. It is statistically limited by the quality of the coronary restoration: temporary or permanent. If the integrity of the coronary restoration is not disturbed, then canal obturation does not play a role in the coronary bacterial leaking. After the period of 5 days the temporary sealed cavities are contaminated with bacteria and the penetration is on the orificium level.

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