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The Reaction of Dental Tissues to the Protective Adhesive Materials Used in the Treatment of Deep Caries

Dentin-pulp complex protection during deep caries treatment is a basic principle in dental practice and involves the use of specific materials as liners or bases. This study aims to determine dental tissue reaction consecutively applying a classic adhesive in deep cavities in comparison with calcium hydroxide which is the biological material of choice for the protection of the dental pulp. Histopathological images show the presence of significant vascular and cellular changes occur in the pulp tissue in case of dentin adhesive used as protective material compared to defensive reaction of calcium hydroxide generated by deposition of tertiary dentin.

Keywords: adhesives, decay, tertiary dentin, dentin-pulp complex

The morphological characteristics of immature permanent teeth represented by the large pulp chamber and by the increased dentin permeability range require strict measures to protect the dental pulp during treatment of deep caries with protective materials such as liners, base materials, sealants and dentin adhesives [1]. From a biological perspective, these materials cannot completely replace the function of protection offered by the enamel and dentin, but must have as its main objective the integrity conservation of the dentin-pulp complex [2, 3].

Another important factor which depends on the pulp tissue preservation, is the depth of the carious cavity, especially the structure and the thickness of the remaining dentin between the cavity floor and the ceiling of the pulp chamber, known as the “Remaining dentine thickness” (RDT) [4, 5]. It was demonstrated that remaining dentin thickness, considered as adequate for the obtaining of a biological restoration by protecting the pulp organ, is 2 mm even in the case of an application of materials that are potentially toxic to the pulp [2].

The introduction of dentin adhesives in dental practice as means of protection is based on forming of a hybrid layer at the molecular level, that is, forming an area with physical-chemical properties that are different from the original structure of the dentin tissue, by the partial demineralization and the impregnation of the exposed collagen fibers with the polymers from the adhesive resins [6-8]. The hybrid layer protects the pulp with an antibacterial sealing of the tooth-restoration interface, making it possible for the biological restoration of the dental integrity. Giammanco et al. [9] used comparatively in vitro two adhesives, one containing an antibacterial agent, to prove that the antibacterial ability of the dentin adhesive is due to their ability to achieve good adhesion to the surface of the dentin and their ability to obtain a fitting seal of the dentin tubules.

Initially a hypothesis was launched, that the restorative materials themselves are toxic for the pulp and therefore the application of liners and bases that would protect vital dentin is essential to the success of the dental treatment. These recommendations were based on studies that showed that the pulp is sensitive to low pH materials used for restoration [1]. Recent studies however, have launched another hypothesis which believes that the main reason for biological failure of dental restorations is not related to pH or other properties of materials, but rather is due to the reduced ability to seal the tooth-restoration interface, which is resulting in the emergence of bacterial marginal infiltration and hence it triggers the pulp defense reactions [10]. This theory has launched a new concept of therapeutic approach, which involves applying the adhesive materials directly to the dentinal tubules exposed, thus ensuring their deep sealing and a neutralization of the bacterial invasion.

The objective of this study is to track in vivo the reaction of immature pulp tissue, consecutive with the application of two protective materials for the treatment of deep caries in immature permanent teeth, a classic dentin adhesive and a preparation based of calcium hydroxide.

Experimental part

This study was conducted with the approval of the Ethics Commission and the approval of patients / their relatives, who were informed about the purpose and methodology of the workmanship, thus obtaining the written consent of the subjects in the study.

The selection of the study groups: there were selected a total of 20 premolars that were scheduled for extraction for orthodontic purposes. The teeth come from 10 children aged 12-14 which are receiving orthodontic appliances. The selection criteria involved the choice of integrity teeth, without carious processes and without clinical symptoms. On all of the teeth were prepared cavities of low depth (at 2.5 to 3.5 mm below the enamel-dentine junction), in which two types of indirect pulp capping materials were applied:

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- Optibond Solo Plus – a mono component adhesive applied through a total-etching technique (etching of enamel and dentin simultaneously);
- Calcimol LC (Voco) – a Light-curing paste based on calcium hydroxide; a base of glass ionomer, Glass Liner (WP) was applied over it.

There were two study groups formed:
- Group 1: 10 premolars on which deep cavities were prepared, where the Optibond Solo Plus dentine adhesive was applied after the total acid etching;
- Group 2: 10 premolars on which deep cavities were prepared where the Calcium hydroxide Calcimol LC was applied and the glass ionomer as a base (table 1).

In the study group 1, the component adhesive system Optibond Solo Plus was used, applied according to the manufacturer as follows: after etching for 30 s with 37% phosphoric acid and washing it with a jet of water, it was followed by the drying of the cavity with a sterile cotton pellet applied gently, thus avoiding the excessive dryness that would produce dentin dehydration. The application of the bonding was carried out by brushing it on the whole surface of the dentin, followed by drying for 15 min to evaporate the solvent, and then polymerization for 20 s after which the second layer of bonding was applied by repeating the steps described above.

For the study group 2, the capping material was used, specifically Calcimol LC, which is a liner based on calcium hydroxide, applied as follows: after washing and drying the cavity, an applicator was used to apply an uniform layer of 1 mm at the base of the cavity, followed by polymerization for 20 s and on top of this was applied a base of light-curing glass ionomer (Glass Liner). In all study groups, cavities were restored with a light-curing composite, Competence (WP), applied and cured in successive layers.

Deep cavities were prepared distanced 2.5-3.5mm under the enamel-dentine junction. The extraction of the premolars was performed 4-6 weeks after the application of the capping materials, and then the extracted teeth were kept in a 4% formalin solution until the prepping for the histopathological examination. Prior to application of the formalin solution, many orifices were drilled, with a diamond milling apparatus, at the level of the crown and the root to facilitate the penetration of the formalin and the conservation of the pulp tissue structure.

To carry out the sectioning, the teeth were decalcified by immersing them for 4-5 days in EDTA, followed by the inclusion in paraffin and the microtome sectioning. For the histopathologic exam using an optical microscope, the sections obtained were stained with a solution of hematoxylin – eosin, and the interpretation was conducted at a magnitude of 100X, 400X, 1000X.

### Results and discussions

A number of 40 histological slides were obtained from the 20 premolar teeth prepared for the study. Among them, the preparations in which the structures of the dentin-pulp complex highlighted best by adequate conservation and restraint were selected.

- Group 1: in the deep cavities prepared close to the pulp cavity (remaining dentin thickness, RDT <0.5 mm) where the dentin adhesive has been applied, there is a tissue disorganization, with complete disappearance of the odontoblast layer, which is highlighted by a lack of deposits of the tertiary dentine layer. Also at this level, emphasized vascular phenomena can be observed, with vessel dilation, intravascular thrombosis and the emergence of neoformation vessels, that denotes a reversible hyperemic inflammation, resulting from the defensive reaction of the pulp tissue to the physico-chemical aggression. Specifically it is the emergence of a lympho-plasmocytor inflammatory infiltrate as a response of the immune defense (fig.1).

There were also revealed the following phenomena:
- Group 2: the results show that tissue changes of the pulp organ is a normal defensive reaction to the physical-chemical aggression. This is evidenced by the appearance of hyperemic vascular reaction and by the emergence of intravascular thrombosis, due to reduced circulation flow.
and a thickness greater than that of the study group. In intense deposits of tertiary dentin uniform in appearance most varied and significant tissue changes could be seen. was applied as a capping material; it is also where the significant histo-pathological changes of the pulp when adhesives are used directly on the dentin, result in irritation, and consecutively, restorations in which other authors state that the acid used for etching causes effects in the same way as do other materials which in deep cavities do not produce pulp damage or other toxic chemical agents. In vitro the effect on the pulp cells produced by the 18 chemical agents such as sodium hypochlorite, zinc oxide, lidocaine, EDTA and have showed that the occurrence of cellular apoptosis phenomena and the genetic changes in the structure of the five genes associated with pulp functions occurs regardless of the cytotoxic effect of chemical agents. Some authors consider that the acid etching performed in deep cavities do not produce pulp damage or other toxic effects in the same way as do other materials which include in their composition dentin adhesives [14-17], but other authors state that the acid used for etching causes irritation, and consecutively, restorations in which adhesives are used directly on the dentin, result in significant histo-pathological changes of the pulp when the thickness is less than 1 mm RDT [18-21]. This can also be observed in our study group 1 where the dentin adhesive was applied as a capping material; it is also where the most varied and significant tissue changes could be seen. In the case of group 2, there was an intense activity of intense deposits of tertiary dentin uniform in appearance and a thickness greater than that of the study group 1. In general, the reaction dentin is secreted by pre-existing odontoblasts, and the repair dentin is secreted by the newly differentiated odontoblasts [7, 14, 22-25]. Wegehaupt et al. [26] have shown in a study conducted in vivo without histological studying of the dental pulp reaction, that after six months, the onset of pain and hypersensitivity are not associated with the thickness of the remaining dentin remaining at the base of the cavity, nor with the type of material used for indirect pulp capping, whether it is calcium hydroxide or dentin adhesives.

Conclusions
The deep carious cavities with a low layer of dentin between the base of the cavity and pulp chamber leave the pulp tissue poorly protected by chemical effect of dental materials. Pulp reaction, when dentin adhesives are used, depends on the depth of the cavity, the type of adhesive and the application technique used in applying it.

Treatment success for the deep decay in immature permanent teeth is primarily represented by the preservation of a dentine layer of at least 1.5-2 mm between the base of the cavity and the pulp chamber and secondarily by the choice of biological protective materials.

Acknowledgments: This work has been developed within the projects 28/2013 funded by the University of Medicine and Pharmacy of Tîrgu Mureş, Romania.

References

Manuscript received: 18. 09. 2015