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Original article

MICROBIOLOGICAL RESEARCH OF *S.MUTANS* DURING THE COURSE OF A MICRO-INVASIVE STEPWISE TREATMENT OF DENTINE CARIES

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ABSTRACT

Introduction: The stepwise, controlled excavation is a method for a micro-invasive treatment in the cases of deep dentine caries, and is carried out in two stages.

Aim: To study the quantity of *S.mutans* during the course of a micro-invasive, stepwise treatment of dentine caries.

Materials and Methods: Subject of the study were 60 permanent children's teeth with dentine carious lesions, which were treated with a stepwise excavation (a three-month course), controlled through a fluorescent method (Proface), distributed into three groups: control group with a complete excavation down to sound dentine and 2 test groups: test group 1 - with excavation down to partially infected dentine, test group 2 - with excavation down to affected dentine. Microbiological identification and quantitative assessment were carious out through a culture study.

Results: After a three-month treatment of partially infected dentine, an additional excavation is necessary in some of the cases, while no bacterial growth is observed in excavated caries lesion to affected dentine. The micro-invasive approach with a stepwise controlled excavation proves the possibility for arresting the cariogenic process and absence of residual cariogenic microorganisms after a three-month treatment.

Keywords: stepwise excavation, dentine caries, *S.Mutans*,

INTRODUCTION

Caries in the dentine is considered a dynamic process, the progression of which depends not only on the microbial invasion and the conditions in the oral environment but also on the pulp's response. The stepwise, controlled excavation is a method for a micro-invasive treatment in the cases of deep dentine caries and is carried out in two stages. With it, there is the potential for arresting the cariogenic process in the dentine, while even preserving partially infected dentine, as well as the possibility for arresting the microbial activity and stimulating the pulp's response [1, 2, 3].

The positive results from the application of a stepwise excavation are demonstrated in a number of studies. Time and the possibility of activating the natural defence mechanisms of the pulp-dentine complex are given. During the first visitation, a partial excavation is performed, and only the most strongly infected dentine in the surface layer is removed. What follows is temporary obturation of the cavity with a method that facilitates internal remineralization and pulp regeneration (most often with calcium hydroxide cement, glass ionomer cement - GIC) and a next visit, control and additional excavation after three to six months, if necessary. Regardless of the fact that most authors use the term "stepwise" excavation, most frequently the excavation procedures of the carious dentine are only two [5, 6, 7, 8].

The methodology of stepwise excavation is described and thoroughly studied by Bjorndal L. in many of his publications [9, 10, 11, 12, 13]. His studies show that there is a significant decrease in the quantity and diversity of species of microorganisms in deep dentine lesions, in comparing the two stages of the treatment [10, 11, 12]. Most significant is the decrease in the number of cariogenic microorganisms – *S.Mutans, Lactobacillus spp*., which the authors attribute to the cutting of access to nutrients and changes in the conditions of the environment [15].

Despite increased scientific interest, regarding the dynamics of cariogenic microorganisms during the course of a micro-invasive treatment of deep dentine caries, the clinical assessment for determining the line between infected and affected dentine during the course of excavation is still uncertain. It is also uncertain how, on the basis of clinically determinable objective markers, to assess the degree of bacterial invasion of the dentine and the quantity of the main cariogenic species. These questions are resolved through the introduction of the fluorescent method of excavation control, which facilitates detection the residual dentine and is ever more often applied in dental practice.

Aim - To study the number of *S.mutans* during the course of a micro-invasive, stepwise treatment of dentine caries.

Tasks - 1. A quantitative description of *S.Mutans* in a carious, partially infected and affected dentine; 2. Determining the quantities of *S.Mutans* at the end of a micro-invasive, stepwise treatment of dentine caries.

MATERIALS AND METHODS

Subject of the study were 60 permanent children's teeth with dentine carious lesions of children between the ages of nine and thirteen, which were treated with a stepwise excavation (a three-month course), controlled through a fluorescent method (Proface), distributed into three groups, according to the degree of excavation (1 control and 2 test groups).

- Control group – 20 dentine carious lesions (D3b) with a complete excavation down to sound dentine, obturated with calcium hydroxide cement and composite.

- Test group 1 – 20 dentine carious lesions (D3b) with excavation down to partially infected dentine, obturated with calcium-hydroxide layer temporary obturation with GIC (for three months).

- Test group 2 – 20 dentine carious lesions (D3b) with excavation down to affected dentine (turbine and micromotor), obturated with calcium-hydroxide layer temporary obturation with GIC (for three months).

Informed consent was granted for each of the children participating in the research by the parents or guardians, through the filling out written consent forms, in accordance with the requirements of the Commission of Ethics in Scientific Research.

Control of the excavation was carried out through the *fluorescent technique (Proface),* by using the fluorescent control criteria developed by this team with *(Proface).* A Bjorndal et al. scale, modified by this research team, was used for a visual-tactile clinical assessment of the dentine [14]. The results were published in a separate article [15].

- Fluorescent criteria for *infected dentine with Proface* – the fluorescence is with an intense red or dark red colouration and entirely encompasses the carious dentine;

- Fluorescent criteria for *partially infected dentine with Proface* – the presence of red colouration of the fluorescent signal, localized only in limited sections of the zone of the dentine above the pulp, surrounded by sections with pink fluorescence. No fluorescence is observed at the dentine enamel junction (DEJ) and the walls of the cavity, which were excavated down to healthy dentine;

- Fluorescent criteria for *affected dentine with Proface* –the fluorescent signal is with a pink colouration, localized only in separate sections on the bottom of the cavity (at the area of the dentine above the pulp), with no fluorescence being observed in the rest of the cavity.

Microbiological methods

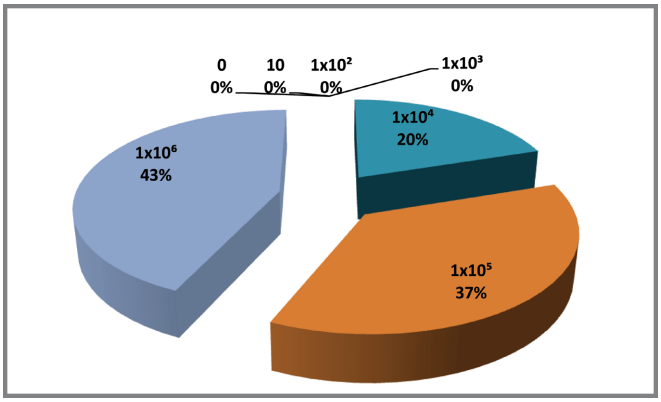
Microbiological identification and quantitative assessment were carious out through a culture study, by the use of the nutritional environments for seeding: blood agar with 5% lamb's blood (CA) 0 Columbia agar, production Becton Dickinson (BD) for isolating streptococci, thioglycolate broth (TG) – Oxoid. The quantitative line method was used for seeding the samples according to a scheme. After cultivation, the developed colonies in the various sectors were counted and compared to a standardized table, by which the microbial count was determined. Biochemical identification was carried out with quick tests for catalase and pyrrolidine amylase and with the REMEL RAPID Strepto ID and Crystal Ana BD identification systems.

RESULTS

2. A quantitative description of *S.Mutans*in a carious, partially infected and affected dentine of the carious lesions

- *Carious dentine* - The data for the quantitative description of *S.Mutans*in the carious dentine are presented in the following diagram.

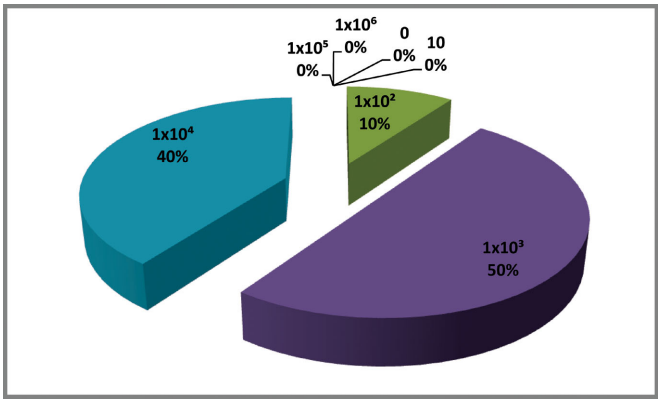
Diagram 1. Quantity of *S.Mutans* in the carious dentine



The results presented show that in 80% of the carious dentine samples tested quantities of *S.Mutans* ≥ $1x10^5$ CFU/ml can be found. A microbial count of these values is considered a significant and severe degree of infection. Clinically this dentine is moist, with a dark or light brown colouration and a soft consistency.

- *Partially infected dentine* - The data for the quantitative description of *S.mutans* in the partially infected dentine are presented in the following diagram.

Diagram 2. Quantity of S.Mutans in partially infected dentine

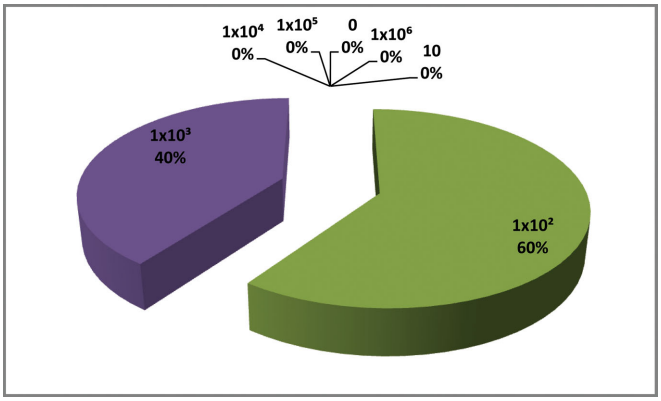


The data shows that in the partially affected dentine predominant (90%) are the cases with quantities of *S.Mutans*of $1x10^4$ and $1x10^3$ CFU/ml. The levels of infection are considered average and borderline and are lower than those registered in infected dentine. Clinically, partially infected dentine is with a dark colour, some mois-

ture and a consistency that allows for the probe to sink.

- *Affected dentine* - The data for the quantitative description of *S.Mutans*in the affected dentine are presented in the following diagram.

Diagram 3. Quantity of S.Mutans in affected dentine.

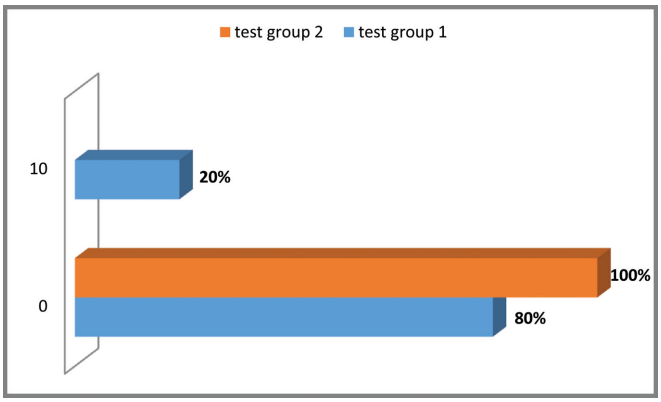


The data shows that in the affected dentine *S.Mutans*can be found in quantities between $1x10^3$ – 40% and $1x10^2$ CFU/ml – 60 %. These levels of infection are considered low. Clinically, this dentine is with a light brown colour, with sometimes more dark brown localized spots and its consistency offers light resistance during probing or is marked by a white trace.

2. Determining the quantities of *S.Mutans* at the end of the micro-invasive stepwise treatment of dentine caries.

After the expiration of the three month treatment period, the second revision of the cases in both test groups was carried out. No fluorescence was present in all cases from test group two, so a second excavation was not necessary. The microbiological results at the end of the treatment showed a lack of bacterial growth in regard to *S.Mutans*. At the end of the treatment, after the expiration of the three month period, 20% of the cases in test group one had a slight, pale pink fluorescence was present. The dentine in separate small sections at the bottom of the cavity was slightly moist and with a slightly soft consistency. A second light excavation of these sections was necessary in these cases.

Diagram 4. Quantity of S.Mutans at the end of the three-month treatment with stepwise excavation



After expiration of the three month period, no bacterial growth was discovered in 80% of the samples from test group1 (with excavation down to partially infected dentine), while in 20% a presence of microorganisms within the quantity of up to 10 units was established. No growth was discovered in any of the samples from the test group (with excavation down to affected dentine) by the end of the period.

DISCUSSION

According to the results of this study, it can be concluded that the stepwise controlled excavation, as a method for micro-invasive treatment of deep dentine caries, shows potential for arresting the cariogenic process, preservation of a larger volume of dentine and the possibility for altering the microbial activity. Proof of this is the change in colouration and consistency of the affected and partially infected dentine after a three month period of stimulation by calcium hydroxide. Studies show that due to the good seal the remaining carious dentine is deprived of sugars – the primary nutritional substrate of microorganisms –the infection becomes weaker, the microbial count is significantly decreased as well as is the variety of cariogenic microorganisms species, which is proven by this study.

The results of this study show that a decreased microbial count of *S.Mutans*is in proportional relation to the hardness of the dentine, its transition to a lighter colouration and a decrease in the intensity of the fluorescent signal. Similar studies have been carried out by other authors before this, and there exists data for in-vivo studies, demonstrating the close relation and proportionality between the consistency and colour of the carious dentine and the number of microorganisms in it. These studies were carried out by tracing clinical cases of deep dentine carious lesions, treated with a stepwise excavation. The microbial count in the cases of hard carious dentine with the light colouration is significantly lower in comparison to that of cases with soft carious dentine with a darker colouration [8, 9, 15, 16, 17].

It was also established in this study that the weak fluorescence present in the affected dentine disappears after the three month period of treatment. In a small number of cases form the group with the partially infected dentine, the presence of a pale pink fluorescence in separate areas of the bottom of the cavity was discovered. It is known that fluorescence in the carious dentine is owed to the presence of bacterial bio-products in it, while the absence of it at the end of the treatment indicates a lack of bacterial growth [18].

The presence of a faint, light pink fluorescence in a small part of the cases from test group 1 (excavation down to partially infected dentine), in combination with the type of residual dentine, necessitated another excavation. The results from the microbiological study at the end of the clinical experiment confirmed this.

The results obtained by us are confirmed by other authors, and in the scientific literature the attention of the researchers is focused on the clinical characteristic of the over pulpal dentin, whose microbial activity is proved by

microbiological research [19, 20].

An in vivo study by Ayse I. Orhan et al. has used clinical and microbiological criteria for determining the effectiveness of stepwise excavation in treating dentine caries. Its results show a 63.8% bacterial growth in the samples from the first procedure of a two-step excavation and 26.6% from the samples of the control group with a complete excavation. Upon the second visitation, after removal of the temporary obturation, the bacterial growth in the two-step excavation samples was reduced significantly, down to 44.4%, while after excavation and before obturation – 2.2%. The results show that despite there being no method to achieve absolute sterility of the bottom of the cavity, the step-by-step excavation is the method, which achieves a significant reduction in the bacterial growth during the course of treatment [17].

E.C.O. Lula et al. conduct a similar study, but on primary molars with dentine lesions, separated into 2 groups – with complete, one-step excavation and a group with partial removal of the carious dentine. The microbiological tests were for *S.Mutans, L. Acidophilus* and also for the total microbial count in the dentine. Before obturation, a larger number of microorganisms were present in the samples of the second group, while after a three to six month period the microbial count and number of colonies of the two species tested for in both groups were similar. The results give a basis for the authors to recommend that a second excavation should not be performed when affected dentine has been reached [19, 20].

The results of this study show that excavation down to partially infected dentine allows for a larger volume of dental structures to be preserved, in which it is possible for the processes of internal remineralization and reactive dentinogenesis and/or reparative dentinogenesis to take place, and the time between both sessions is given with the aim to slow or arrest the cariogenic process and activation of the defence mechanisms. In a single session, dentine carious lesions possessing the characteristics of a slow progression (hard, dry, dark brown or black colouration), may be treated, by excavation down to affected dentine. These lesions are excavated upon the first visitation, down to affected dentine and are obturated permanently.

CONCLUSIONS:

1. The micro-invasive approach with a stepwise controlled excavation proves the possibility for arresting the cariogenic process and absence of residual cariogenic microorganisms after a three-month treatment;

2. After a three month treatment of partially infected dentine, an additional excavation is necessary in some of the cases, while no bacterial growth is observed in excavated caries lesion to affected dentine.

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