Decontamination of dental cavities using solution of chlorhexidine versus two type of mouth water. An "in vitro" study

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Abstract

Aim and objectives. The aim of this study is to evaluate the effect of two types of mouthwash compared to the effect of 0.2% chlorhexidine gel in class I prepared dental cavities.

Material and methods. The study included 10 human molars. Class I dental cavities were created. The samples were inoculated with Streptococcus mutans suspension. After this part, the experimental procedure was applied using three decontamination solutions: chlorhexidine, Listerine Cool Mint mouthwash and Total Care. After treatment, samples were collected for microbiological analysis.

Results. After carrying out the ANOVA and Tukey -Kramer test for Streptococcus mutans values shows that there is a significant difference between the results of group 3, where Listerine Cool Mint was applied, and the results of group 5, where CHX 2% gel was chosen to be applied.

Conclusions. The most significant reduction in the number of Streptococcus mutans, is recorded in group 5, the one in which the 0.2% chlorhexidine gel was applied for 60 seconds.

Keywords: Streptococcus mutans, chlorhexidine, Listerine, dental cavities

INTRODUCTION

Dental caries is one of the most important public health problems and a very widespread disease worldwide. It is an irreversible, chronic, infectious disease that evolves as a dynamic, multifactorial process and affects the mineralized dental structures. Dental caries is a complex disease caused by enamel demineralization and remineralization in the presence of fermentable carbohydrates, saliva, and cariogenic oral flora [1].

Dental caries is a lesion that forms on the surface of the tooth in the form of a cavity that progresses and results in the loss of tooth structure. Demineralization causes initial changes at the ultrastructural level, which can only be observed with the electron microscope. Clinically, it cannot be detected in its early stages, but as the lesion progresses, the dentist will notice a decrease in enamel translucency, which can be detected during an intraoral examination of the patient. When bacteria accumulate in dental plaque and ferment dietary carbohydrates for a long time and locally produced acids cannot be neutralized by the buffering capacity of saliva, tooth demineralization occurs, leading to cavitary forms of carious lesions [2-5]. Although the ability of low pH to demineralize enamel is well established, tooth decay is a multifactorial disease caused by microbes and influenced by dietary habits, dental characteristics, the buffering capacity of saliva and the host's immune system [5].

Control of the oral microsystem, prevention and treatment of oral diseases is often achieved with the help of antibiotics and antiseptics. The use of these antimicrobial solutions aims to decrease the total microbial load to combat the given condition. One of the most common antiseptics used in oral health care is chlorhexidine (CHX), a bactericidal agent. CHX has a broad spectrum of efficacy and works by interfering with the cytoplasmic or inner wall of the bacterium once it has successfully crossed the outer membrane. CHX is usually added in treatment variants at a concentration of 0.12 or 0.2%. Both concentrations are well above the minimum inhibitory concentration (MIC) of the oral strains tested [6]. However, such tests expose the microorganisms to a constant concentration of the bactericide. Although this approach is a viable solution for systemic treatment, it is not representative of a topical treatment such as mouthwash application. The contact time between the bacteria and the antiseptic is between 60 and 90 seconds during the treatment performed in the oral cavity. Moreover, in the case of oral conditions, the target is a biofilm polymicrobial, and bacteria in biofilms show increased tolerance to antimicrobials compared to planktonic bacteria. Finally, biofilm architecture can greatly influence treatment outcome. The outer layers of the biofilm are more susceptible to antiseptic compared to the inner layers [7,8].

Aim and objectives

The aim of this study is to evaluate the effect of two types of mouthwash compared with the effect of 0.2% chlorhexidine gel in class I prepared dental cavities. Knowing an effective bacterial decontamination protocol at the level of prepared dental cavities will be of real use for the dentists, because a much better and risk-free prevention will be achieved after the long-term obturation with composite materials. All this, competing to significantly reduce the risk of developing residual carious lesions on the long term.

At the beginning of the study, the null hypothesis was established, that there is no statistically significant difference between the three types of treatments used within the experimental groups.

MATERIAL AND METHODS

The work protocol was reviewed and approved by the Research Ethics Committee of the "Victor Babeş" University of Medicine and Pharmacy in Timişoara.

The participation of the patients in the study to collect biological samples, was conditional on being informed both orally and in writing, regarding their role in the research, also, the informed consent was obtained after being informed and understanding by each participant of all the information regarding the study and the way it is conducted.

Inclusion criteria were as follows: teeth whose coronal part was intact.

The exclusion criteria were the following: coronary fillings, clinically detectable carious lesions, white spot lesions, previous exposures to chemical treatments such as hydrogen peroxide, and changes caused during extractions by elevator or forceps, respectively.

The study included 10 human molars extracted for orthodontic/periodontal reasons, and which were kept for a maximum of 30 days in a 0.9% sodium chloride solution at 23◦C, until the beginning of the experiment.

After choosing the samples, they were cleaned by ultrasonic scaling (using EMS miniPiezon, SA CH-1260 Nyon Swiss) and professional brushing with fluoride-free paste (Clean Polish, Kerr Hawe).

The dental samples were randomly distributed in the following experimental groups (Fig. 1), as follows:

1. Positive control group
2. Negative control group
3. Listerine Cool Mint
4. Listerine Total Care
5. Chlorhexidine gel 0.2%

Figure 1. Highlighting the experimental groups selected in the study

Occlusal surface of each tooth was prepared to obtain a Class I cavity with a globular/pear diamond instrument on the high speed (turbine) handpiece.

To have a better control over the preparation of class I cavities, 3 mm was measured with a periodontal probe, at the level of which a rubber stopper was applied, thus facilitating the stage of periodic verification after each moment of dental preparation (figure 2).

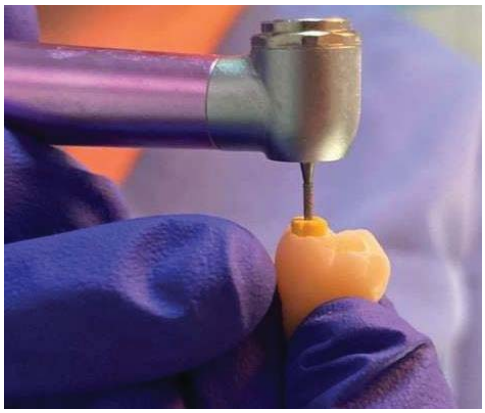
a.  b. 

Figure 2. Cavity depth preparation controlled by fixing the rubber stopper on the diamond instrument at 3 mm (a) and the dental cavities prepared on all samples (b)

After making the cavities, the samples were sterilized to ensure the absence of any bacterial form, in an autoclave (C306552 ZETACLAVE B 231, Zhemark S.p.A. B class, Italy) at 121◦C for 20 min; then, samples were collected from the cavities obtained, using a sterile applicator that was stored in a sterile container with broth liquid and placed in a thermostat (Jouan IG150 Infrared-controlled CO 2 Incubator, Germany) for 24 hours at 37◦ C, time required for the development of a possible existing bacterial form. After the 24 hours, the bacterial forms were cultivated on a culture medium with blood agar (Columbia Agar + 5% ram blood, Mediclim, Romania) for 24 hours at 37◦C in the same thermostat (Fig. 3), which will show us whether forms of microorganisms were present in the cavities created, after sterilization.

Figure 3. The stage of harvesting and seeding from the level of dental samples after the time of sterilization

An ATCC 25175 suspension containing the S. mutans reference strain was prepared at 0.5 McFarland units [9] using a densitometer (DEN-1 McFarland Densitometer, Biosan, BS-050102-AAF, LATVIA).

After preparing the bacterial suspension, 8 of the 10 samples (except the positive control group that was not inoculated) were stored in a sterile container. 20 ml of liquid synthetic culture medium (broth) was placed in each container and 280 µl were taken from the Streptococcus mutans (S.m.) suspension with the microbiological pipette and mixed with the broth in which the samples were stored. At the end of the process, all containers were inserted into the same thermostat for 24 hours at 37°C to obtain microbial colonies at the level of the prepared odontal cavities.

The next day, from each inoculated container, samples were taken for inoculation on the agar-blood culture medium, to be stored at a thermostat for 24 hours at 37°C to observe the existence of microbial colonies (Fig. 4).

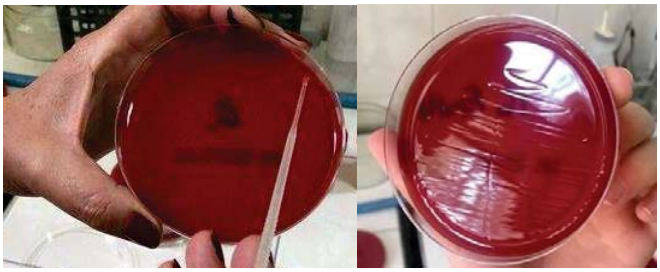


Figure 4. Inoculation of samples on agar-blood culture medium taken from containers inoculated with bacterial suspension

To apply the treatment of group 3, the solution of Listerine Cool Mint (Johnson & Johnson Pvt.Ltd. USA) was used (Fig. 5). The solution was applied in the prepared cavity of the two dental samples, leaving it to act for 60 seconds, after which it was removed by washing for 5 seconds with the water-air syringe from the dental unit and drying with a sterile pad. For all two samples, the treatment consisted of a single application.

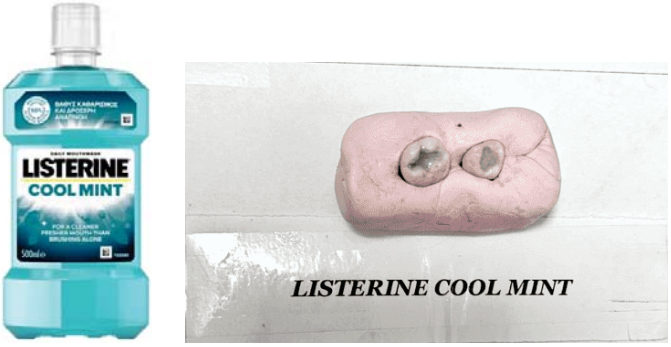


Figure 5. Application of the Listerine Cool Mint solution in the class I cavity at the level of the two dental samples of the experimental group 3

To apply the treatment of group 4, the Listerine Total Care solution (Johnson & Johnson GmbH, D-41470 Neuss, DE) was used (Fig.6) in the prepared cavity of the two dental samples, leaving it to act for 60 seconds, after which it was removed by washing for 5 seconds with the water-air syringe from the dental unit and drying with a sterile pad. For all two samples, the treatment consisted of a single application.

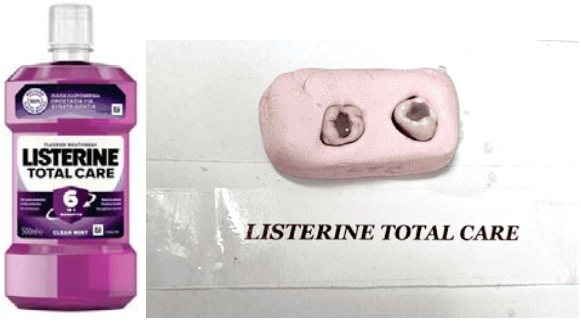


Figure 6. Application of the Listerine Total Care solution in the class I cavity at the level of the two dental samples of experimental group 4

To apply the treatment of group 5, gluconate of 0.2% chlorhexidine gel (Elugel 0.2%, Oral Care, France) was used (Fig. 7). The gel was applied in the dental cavity at the level of

both dental samples, leaving it to act for 60 seconds, after which it was removed by washing for 5 seconds with the water-air syringe from the dental unit and drying with a sterile pad. For both samples, the treatment consisted of a single application.

Figure 7. Application of 0.2% CHX gel in the class I cavity at the level of the two dental samples of experimental group 5

After applying the treatment, samples were taken from the dental cavities of experimental groups 3, 4 and 5 with sterile applicators and placed in the broth solution, at a thermostat for 24 hours, 37°C. After this 24-hour period, culture media were seeded with samples from the broth solution and placed in a thermostat for 24 hours at 37°C.

After the two stages of harvesting and seeding, the microbial colonies on the culture media were observed and counted from the 3 moments of harvesting: after the sterilization stage, before applying the treatment with the S.m. suspension. applied to the level of the dental samples and after applying the treatment.

Statistical analysis was performed using Microsoft Excel (version 2012 for WINDOWS), one-way analysis of variance (ANOVA) and the Tukey -Kramer test.

RESULTS

According to several microbiological researches, the numerical results were recorded following the evaluation of each examined sample using a digital colony counter (Colony Star, MultiLab, Funke Gerber), as can be seen in Table 1 and to simplify colony counting a score will be assigned to each numerical value obtained: score 0 is given to the absence of colonies or less than 10 CFU/ml, score 1 = 10^2 CFU/ml, score 2 = 10^3, score 3 = 10^4, score 4 = 10^5, score 5 = 10^6, score 6 = > 10^7 CFU/ml, [10].

Table 1. Numerical values of the microbiological evaluation in the 3 moments of harvesting

	After sterilization of dental specimens	After the inoculation of SAMPLE with the suspension of S. m.	After experimental treatment		Mediate	SD
			MINIMUM VALUES	MAXIMUM VALUES		
Gr 1 – positive control	0	0	0	0	0	0
Gr 2 – negative control	0	6	6	6	6	0
Gr 3 – Listerine Cool Mint	0	6	4	4	4	0
Gr 4 – Listerine Total Care	0	6	3	4	3.33	0.52
Gr 5 – CHX 0.2%	0	6	1	2	1.50	0.58
SD – standard deviation						

After applying various decontamination solutions to reduce the number of S.m., lower values resulted, compared to those before the treatment, which can be seen in figure 8.

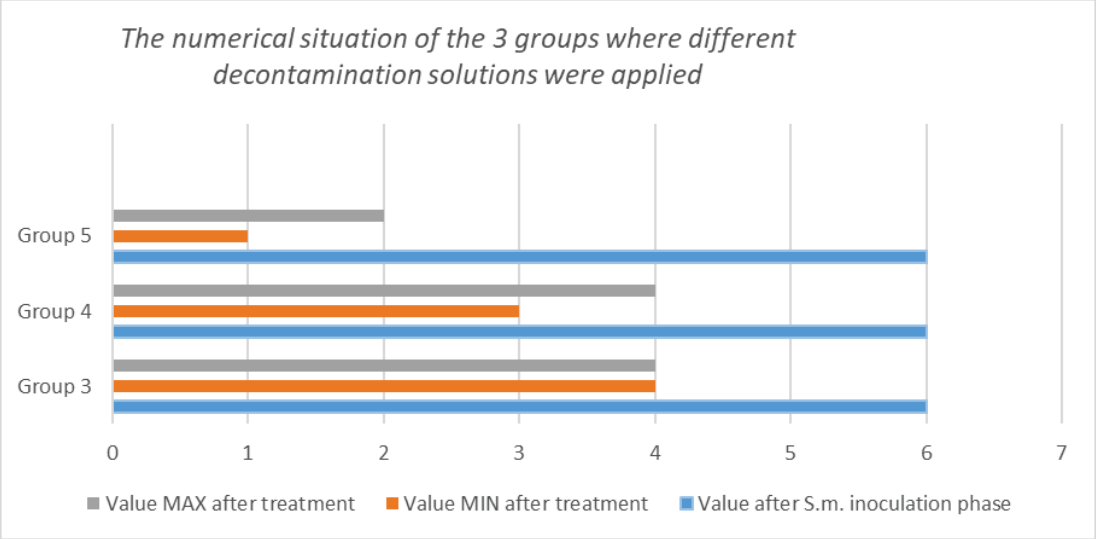


Figure 8. Evolution of the number of S.m. colonies in different stages of the study

For this "in vitro" study, one-way analysis of variance (ANOVA) was used, and depending on the numerical results recorded, the Tukey-Kramer test should be applied to establish possible differences between the types of treatments applied. The level of statistical significance was set at 5% ($p<0.05$), and the confidence level at 95%.

As can be seen in table 1, for group 1, positive control, the values being identical, the mean and standard deviation is zero. The same situation regarding the recording of a zero standard deviation is found in group 2 and 3.

The highest mean of the values comes from group 2 where no treatment was applied, this being considered a negative control, at the level of which only the inoculation of the dental cavities with S.m. suspension was carried out, while the lowest mean is observed in group 5 where applied CHX 2% gel.

Following the one-way ANOVA analysis, a significant difference can be observed between the experimental groups, regarding the numerical values recorded after the experimental moments ($p<0.05$).

After carrying out the Tukey-Kramer test for S.m, values shows that there is a significant difference between the results of the groups in which the experimental treatment was applied, compared to the control group samples ($p<0.05$), values that can be observed in Table 2. It can also be observed that there is also a statistically significant difference between the results of group 3, where Listerine Cool Mint was applied, and the results of group 5, where CHX 2% gel was chosen to be applied.

Table 2. Numerical values of the microbiological evaluation in the 3 moments of harvesting

Group vs.	*Group*	*Average Difference (%)*	*The standard error*	*Sig.*
1	2	6	0.05	1
3	1	4.11	0.05	1
3	2	-0.6667	0.05	0
4	1	3.33	0.05	1
4	2	-2.2221	0.05	1
4	3	1.5	0.05	0
5	1	1.5	0.05	1
5	2	-3.33	0.05	1
5	3	-1.1667	0.05	1
5	4	1.55	0.05	1

From Table 2 we can see that there is a statistically significant difference for the compared pairs whose Sig is 1, and otherwise, Sig = 0, we do not have a statistically significant difference. Values of Sig = 1 appear for groups 3, 4 and 5 compared to group 1. Also, a statistically significant difference results from the comparison between group 5 and group 3 and 4, where the 3 decontamination solutions were applied. Comparing group 3 with 4, where the Listerine Cool Mint solution was chosen, respectively Total Care, no statistically significant differences were recorded.

DISCUSSIONS

The aim of this study was to evaluate the effect of two types of mouthwash compared to the effect of 0.2% chlorhexidine gel on prepared Class I dental cavities. Knowing an effective bacterial decontamination protocol at the level of prepared dental cavities will be of real use to dentists, because a much better and risk-free prevention will be achieved after the long-term obturation with composite materials. All this, competing to significantly reduce the risk of developing residual carious lesions in the long term. For this purpose, S.m. colony counts were performed and the distribution of colonies in culture media was interpreted.

The recorded results show the achievement of a significant reduction of S.m. colonies after the application of CHX 0.2% gel compared to the two types of Listerine used, therefore, the null hypothesis was stated that there are no statistically significant differences between the types of solutions oral decontamination, may be rejected.

The results of the present study, obtained after the application of CHX 0.2% gel are consistent with the results of other studies in the specialized literature, demonstrating its ability to reduce bacterial plaque at a concentration of 0.2% [11].

The efficacy of alcohol-free Listerine as a factor in reducing plaque and gingivitis compared to the placebo group (a 5% hydro-alcohol mouth cream) was first demonstrated by Charles et al. [12] in a single-center, randomized, examiner-blind, two-week, no oral hygiene, parallel-group, controlled clinical trial of 90 participants. Listerine without alcohol was more effective ($p < 0.001$) than the negative control group in reducing plaque, reducing gingivitis, and reducing gingival bleeding.

Ulkur et al. [13], evaluating the number of bacteria, shows that after using an alcohol-free essential oil mouthwash for four days, the number of Streptococcus mutans colonies is slightly higher compared to alcohol-based essential oils and water mouth with 0.1% alcohol-free CHX, while no difference appeared on the surface of the tongue.

Traditional essential oils containing alcohol have been used for years as tooth brushing adjuncts in the oral hygiene approach, so their effectiveness in controlling plaque and gingivitis is well documented in the literature; appeared equivalent to CHX for long-term control of gingival inflammation, but CHX appears to perform better than essential oil and alcohol mouthwashes in plaque control [14].

This "in vitro" experimental study aimed to observe the effectiveness of several types of treatments to reduce pathogenic oral microorganisms in class I dental cavities, areas of major importance that will later be filled with coronary restorative materials and where there is most often the risk of developing residual carious lesions because of an incorrect toilet of the cavity.

Although the three treatments gave good results in this research, the best efficiency is demonstrated by group 5, where the CHX 2% gel was applied, compared to the other groups, less so with group 3 whose treatment was based on Listerine Cool Mint, where the results did not show statistically significant differences with the negative control group.

Individual studies have shown that 0.2% CHX mouthwash can reduce plaque indices and the incidence of white spot lesions from fixed orthodontic appliances [15]. However, a

systematic review by Tang et al. [42], although detecting significant reductions in S. Mutans with CHX mouthwash, found only little evidence that CHX use is related to clinical benefits with reduced carious lesions for people wearing fixed orthodontic appliances [16].

Currently, for the prevention of carious lesions, daily oral rinsing with 0.05% sodium fluoride is recommended [17-21]. The results of these studies in the specialized literature are not consistent with the numerical values recorded after the application of the 0.2% CHX gel, which reveals the fact that the clear effect and mode of action of the CHX gel in concentration is not known now of 0.2% at the level of the dental hard structure.

Although the obtained results can guide us towards the introduction into the daily practice of odontal treatments, of a protocol for decontamination of cavities prepared with Listerine solution or CHX 0.2% gel as the main agent, the limits of this study can refer to the number restricted by dental samples, the use of the same decontamination solutions but with their application in the prepared cavities for a period longer than 60 sec, or their use in combinations, gel-solutions, as well as the implementation of another evaluation method, with fewer human errors.

CONCLUSIONS

1. The most significant reduction in the number of Streptococcus mutans, is recorded in group 5, the one in which the 0.2% chlorhexidine gel was applied for 60 seconds.

2. Dental treatments are carried out throughout the dental practice, which will determine an appropriate implementation from this point of view of the appropriate use of chlorhexidine, as a protocol for decontamination of dental cavities, reducing the risk of developing residual carious lesions.

3. The most accessible adjunctive method to tooth brushing and widely available, at a moderate price, is the use of CHX in a concentration of 0.2%, with satisfactory results regarding the reduction of the number of pathogenic oral microorganisms depending largely on the period of use and patient loyalty from this point of view.

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