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Bacterial Flora in Dental Cavities after Traditional and Alternative Methods for Cavity Preparation

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Abstract

The aim of the study was to establish the qualitative and quantitative composition of microbial flora of dental cavities (DCs) after traditional and alternative preparation, including different methods of isolating the working field.

Material and Methods: Our study included 60 patients (mean age of 25.0±3.1 years) with DC Class 1 (Black's classification) without concomitant somatic pathology. To accomplish the study's aim, 60 teeth were prepared. The main group (MG) consisted of 45 teeth prepared under absolute isolation with a rubber dam (RD). In the MG, 15 teeth were treated traditionally with a diamond bur with red and yellow markings (MG-1), 15 teeth were treated by ultrasound with a diamond tip (MG-2), and 15 teeth underwent hydrokinetic preparation (MG-3) with the Aquacut device (Velopex). In the comparison group (CG), which included 15 teeth, DCs were treated traditionally with a dental bur without the RD. The MG and CG were comparable in terms of the initial state of dental and microbiological status. The study of the qualitative and quantitative composition of the DC microflora showed that all DCs contained pathogenic β-hemolytic streptococcus in the CG. At the same time, the maximum number of cases (80%) was moderately contaminated. MG-1 and MG-2, as in the CG, were characterized by the predominance of β-hemolytic streptococcus at the bottom of the treated cavity. At the same time, the incidence of moderate contamination decreased by 4 times and single contamination increased in the cultures to 80%, compared to the CG (P=0.001). In MG-3, β-hemolytic streptococcus also dominated in the bottom of DCs after RD setting. The number of colonies was single (66.7% of cases) and moderate (33.3% of cases), indicating a significant increase in single and a decrease in moderate infestation, compared to the CG (P<0.01). Analysis of the quantitative characteristics of the microbial composition of the cavity floor after preparation of fissure caries showed the highest bacterial contamination in CG: β-hemolytic streptococcus predominated, averaging 251.20±2.5 CFU/tampon. Lactobacillus and Neisseria spp. were detected much less frequently (3.16±1.6 CFU/tampon and 1.99±1.3 CFU/tampon, respectively). There was a 10-fold decrease in the number of β-hemolytic hemolytic streptococcus cultures in MG-1 (25.12±2.0 CFU/tampon), MG-2 (25.12±2.0 CFU/tampon) and MG-3 (19.95±2.0 CFU/tampon), compared to the CG (P=0.000). The opportunistic microorganisms in the treatment of hard tissues by different methods (burr, ultrasound, hydrokinetic) under absolute isolation conditions were identified in almost equal numbers, with the Lactobacillus contamination being significantly lower in MG-1, MG-2, and MG-3 than in the CG (P<0.01).

***Conclusion*:** After the preparation of DCs, a single presence of opportunistic microorganisms, moderate or single contamination with pathogenic bacteria, and absence of anaerobic bacteria were noted. Absolute isolation with RD provides a reduction of microbial infection regardless of the preparation method, and the maximum positive effect is DC preparation with dental burr and ultrasound. (**International Journal of Biomedicine. 2022;12(3):412-416.**)

Keywords: bacterial flora • tooth preparation • dental bur • ultrasound • hydrokinetic method • rubber dam

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Abbreviations

CFU, colony-forming units; **CFR**, caries filling ratio; **DC**, dental cavity; **OHI-S,** the simplified oral hygiene index; **PMA**, papillary-marginal-alveolar index; **RD,** rubber dam.

Introduction

The prevalence of dental caries is very high worldwide, reaching 99%.[1] Treating caries consists of removing damaged tissues and replacing them with fillings. The caries treatment is the most time-consuming stage and depends on the localization of the dental cavity (DC), the extent of the lesion, the tooth group membership, the oral hygiene, the properties of the filling material, and the patient's aesthetic requirements.[2-4] Improving the efficiency of preparation is an important issue in modern dentistry.[5]

Currently, rotary instruments (burrs, cutters) have not undergone fundamental changes. However, the principle of gentle preparation of hard tissues is increasingly being promoted.[6-8] In recent decades, the ultrasonic method, with the use of special nozzles with diamond crumbs of various shapes and sizes, is widely used for tooth preparation, which allows us to reduce the working pressure of the tip and heating of the tooth.[9] It has been demonstrated that the DC treatment by ultrasound removes only softened demineralized enamel and dentin and does not affect the healthy tissues of the tooth.[7] Another alternative method of preparation is hydrokinetic, which is based on the mechanical effects of the flow of aluminum oxide crystals, accelerated by compressed air.[10] Including water in the process minimizes dust formation and increases cutting efficiency. The tissue cutting becomes cleaner and softer than with other preparation methods. Minimally invasive treatment with hydrokinetic preparation does not require local anesthesia, does not cause overheating, and maximally preserves healthy tooth tissue.[11] An important advantage of the method is that a lubricated layer does not form after treatment: under the influence of an abrasive jet, a rough surface free from technical and organic contaminants with maximum contact area is formed.[10,11]

The numerous adverse effects of conventional preparation, in addition to mechanical and thermal trauma, also include microbial invasion.[12,13] The presence of infected dentin and oral contamination make the burr the main carrier of cross-infection.[6] The disadvantages of traditional preparation have led to the search for new types of dental hard tissue treatment that minimize the violation of the microstructure and mineral exchange, as well as microbial contamination.

The aim of the study was to establish the qualitative and quantitative composition of microbial flora of DCs after traditional and alternative preparation, including different methods of isolating the working field.

Material and Methods

Our study included 60 patients (28 men with an average age of 25.43.39±3.21 years and 32 women with an average age of 24.53±3.04 years) with DC Class 1 (Black's classification) without concomitant somatic pathology. To accomplish the study's aim, 60 teeth were prepared. The main group (MG) consisted of 45 teeth prepared under absolute isolation with a rubber dam (RD). The RD was made of latex or nitrile (for latex-allergic patients) flexible cloths (5×5 cm or 6×6 cm). In the MG, 15 teeth were treated traditionally with a diamond bur with red and yellow markings (MG-1), 15 teeth were treated by ultrasound with a diamond tip (MG-2), and 15 teeth underwent hydrokinetic preparation (MG-3) with the Aquacut device (Velopex). In the comparison group (CG), which included 15 teeth, DCs were treated traditionally with a dental bur without the RD.

All patients of the MG and CG were examined using standard diagnostic methods to assess the dental status: the level of oral hygiene by OHI-S index (Greene-Vermillion, 1964), the condition of teeth by the intensity of caries using CFR [the sum of carious, filled, and removed teeth], the condition of periodontal tissues taking into account clinical signs, and an index assessment of the severity of the inflammatory reaction using the PMA index in modifications of C. Parma (1960).

To assess the local microbiological status, all patients underwent a preliminary microbiological examination (β-hemolytic streptococcus, Lactobacillus, Neisseria spp., anaerobes) of the oral cavity. In the morning, on an empty stomach, a smear was taken with a sterile cotton swab from the mucous membrane of the cheeks and palate, followed by sowing on standard nutrient media and identification of the studied microorganisms. Anaerobic culture dishes were incubated in GenBox containers under anaerobic conditions for 24-72 hours at 37°C. Bacteriological examination for the isolation of aerobic and anaerobic microorganisms was carried out with mandatory quantification of the results, which is necessary for the isolation of opportunistic bacteria. After counting the number of isolated colonies on dense nutrient media, the isolated cultures were identified. A complex of morphological, cultural, and biochemical characteristics was used to identify the type of isolated bacteria. Biochemical identification of pure cultures was performed using a bacteriological analyzer. The population density of different groups of microorganisms was expressed in CFU.

The MG and CG were comparable in terms of the initial state of dental and microbiological status. In the oral cavity in patients of both groups, no visible pathology of the mucous membrane or periodontal disease was noted. The PMA index was equal to 0 in all the studied groups, which indicated the absence of inflammatory changes in the soft tissues of the oral cavity. The CFR ranged from 5 to 10. The state of oral hygiene in patients of both groups was characterized as good (the OHI-S index of up to 0.6). All patients in the study groups were characterized by the microbiologically confirmed presence of β-hemolytic streptococcus in the oral cavity (Table 1).

Statistical analysis was performed using STATISTICA 10.0 (Stat-Soft Inc, USA). For descriptive analysis, results are presented as mean±standard error of the mean (SEM). A non-parametric Mann-Whitney U-test was used to compare the differences between the two independent groups. The frequencies of categorical variables were compared using Pearson's chi-squared test or Fisher's exact test (2-tail), when appropriate. A probability value of $P<0.05$ was considered statistically significant.

Table 1.

Clinical characteristic of the groups.

Parameter	CG (n=15)	MG-1 (n=15)	MG-2 (n=15)	MG-3 (n=15)
Average age, years	25.40±3.36	23.67±2.58	25.93±3.24	24.80±3.12
Gender, male/female	7/8	7/8	7/8	7/8
The presence of concomitant diseases, yes/no	no	no	no	no
The initial state of the oral cavity: presence of β-hemolytic streptococcus,yes/no	yes	yes	yes	yes
Dental status: CFR, OHI-S PMA index, %	7.19±1.55 0.54±0.16 0	6.82±1.52 0.52±0.15 0	7.82±1.61 0.57±0.17 0	7.46±1.59 0.58±0.17 0

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee at the Scientific Centre for Family Health and Human Reproduction Problems (Irkutsk, Russia). Written informed consent was obtained from all participants.

Results and Discussion

The study of the qualitative and quantitative composition of the DC microflora showed that all DCs contained pathogenic β-hemolytic streptococcus in the CG. At the same time, the maximum number of cases (80%) was moderately contaminated (up to 103CFU) (Table 2). Lactobacillus was detected much less frequently, in single (20%) and moderate (6.7%) numbers. Single Neisseria spp. were rare (6.7% of cases). Anaerobic bacteria were not identified in the samples submitted.

Preparation in MG-1, as in the CG, was characterized by the predominance of β-hemolytic streptococcus at the bottom of the treated cavity (Table 2). At the same time, there was a statistically significant decrease in its number, compared to the preparations without latex RD isolation. Thus, the incidence of moderate contamination decreased by 4 times and single contamination increased in the cultures to 80%, compared to the CG (P=0.001). Single opportunistically pathogenic Lactobacillus and Neisseria spp. were observed in 20% and 6.7% of cases, respectively, which was similar to the contamination of DCs treated without absolute isolation. Anaerobes from the bottom of DCs were not detected, as in the previous group.

In MG-2, the quantitative and qualitative composition of the cavity floor microflora was identical to that in MG-1: a lower moderate and increased single β-hemolytic streptococcus contamination, single colonies of Lactobacillus and Neisseria spp. (in 20% and 6.7% of cases, respectively), and no anaerobic bacteria.

In MG-3, β-hemolytic streptococcus also dominated in the bottom of DCs after RD setting. The number of colonies was single (66.7% of cases) and moderate (33.3% of cases), indicating a significant increase in single and a decrease in moderate infestation, compared to the CG (P<0.01). There were no significant differences, compared to MG-2 and MG-1. There was a slight trend toward a 13.3% decrease in Lactobacillus singularly isolated, compared with all previous groups. Neisseria spp. were isolated in 6.7% of cases, which corresponded to a similar figure in other methods of carious cavity preparation. Anaerobic bacteria, as in the previous groups, were not detected.

Table 3 shows the quantitative characteristics of the microbial composition of the cavity floor after preparation of fissure caries by different methods. The highest bacterial contamination was detected when working with a dental burr drill without RD isolation. β-hemolytic streptococcus predominated, averaging 251.20±2.5CFU/tampon. Lactobacillus and Neisseria spp. were detected much less frequently (3.16±1.6 CFU/tampon and 1.99±1.3 CFU/tampon, respectively).

Absolute isolation of the working field with RD during preparation allowed us to reduce significantly the bacterial contamination of DCs. There was a 10-fold decrease in the number of pathogenic β-hemolytic streptococcus cultures in MG-1 (25.12±2.0 CFU/tampon), MG-2 (25.12±2.0 CFU/tampon) and MG-3 (19.95±2.0 CFU/tampon), compared to the CG (P=0.000).

Table 2.

The prevalence of bacterial flora in DCs after preparation by traditional and alternative methods.

Parameter		Bacterial flora							
		β-hemolytic streptococcus		Lactobacillus			Neisseria spp.		Anaerobes
Degree of contamination (CFU/tampon)		Single	Moderate	Single	Moderate	Absence	Single	Absence	Absence
Cavity preparation	CG (n=15)	3(20%)	12(80%)	3(20%)	1(6.7%)	11(73.3%)	1(6.7%)	14(93.3%)	100%
	MG-1 (n=15)	12(80%)*	3(20%)*	3(20%)	-	12(80%)	1(6.7%)	14(93.3%)	100%
	MG-2 (n=15)	12(80%)*	3(20%)*	3(20%)	-	12(80%)	1(6.7%)	14(93.3%)	100%
	MG-3 (n=15)	10(66.7%)*	5(33.3%)*	1(6.7%)	-	14(93.3%)	1(6.7%)	14(93.3%)	100%

* - *statistically significant differences with CG*

Table 3.

Microbial composition and degree of cavity floor saturation after preparation of fissure caries by different methods (in CFU / tampon).

Parameter		Bacterial flora		
Degree of cavity floor saturation (CFU/tampon)		β-hemolytic streptococcus	Lactobacillus	Neisseria spp.
Cavity preparation	CG (n=15)	251.20±2.5	3.16±1.6	1.99±1.3
	MG-1 (n=15)	25.12±2.0*	1.58±0.08*	1.26±0.06
	MG-2 (n=15)	25.12±2.0*	1.58±0.08*	1.26±0.06
	MG-3 (n=15)	19.95±2.0*	1.26±0.06*	1.26±0.06

* - *statistically significant differences with CG.*

The opportunistic microorganisms in the treatment of hard tissues by different methods (burr, ultrasound, hydrokinetic) under absolute isolation conditions were identified in almost equal numbers, with the Lactobacillus contamination being significantly lower in MG-1, MG-2 and MG-3 than in the CG (P<0.01). No significant changes for Neisseria spp. were found between all groups.

Thus, after preparation of DCs without RD, the single presence of opportunistic microorganisms, moderate or single presence of pathogenic microflora, and absence of anaerobic bacteria were noted. To a greater extent, microbial contamination was influenced by the method of isolating the working field. Minimal infection was observed in absolute isolation with RD. During hydrokinetic preparation, only a tendency to a decrease in the number of pathogenic β-hemolytic streptococci was noted, compared with teeth treated with dental burr and ultrasonic diamond tips under identical isolation conditions. The presence of pathogenic β-hemolytic streptococcus in the prepared DCs may be due to the etiology of the disease.[13,14] The predominance of moderate contamination at work without RD indicates the entry of microorganisms from infected oral fluid and with oral respiration. The absolute predominance of aerobic coccus flora in fissural caries is confirmed by the works of other authors.[11,14,15] The combined detection of opportunistic and pathogenic microorganisms confirms their ability to combine into microbial associations that contribute to virulence.[11]

Thus, an oral cavity is a place where there are all the conditions for optimal life activity of resident bacterial microflora, and in DCs, there are all the conditions for the favorable existence and activity of not only aerobic but also anaerobic microflora.[16] Microbial contamination of the oral cavity with caries significantly increases in all parts of the mouth, including DCs.[12-14,17] However, it should be noted that not all authors are of the opinion that certain representatives of the resident microflora are particularly cariogenic and give paramount importance to the nature and characteristics of the relationship between the resident microflora and the organism.[18,19]

Conclusion

After the preparation of DCs, a single presence of opportunistic microorganisms, moderate or single contamination with pathogenic bacteria, and absence of anaerobic bacteria were noted. Absolute isolation with RD provides a reduction of microbial infection regardless of the preparation method, and the maximum positive effect is DC preparation with dental burr and ultrasound.

Competing Interests

The authors declare that they have no competing interests.

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