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DETERMINATION OF THE TERMS OF DIFFUSION OF CHLORHEXIDINE ACETATE FROM THE FILLING MATERIAL "RESTAVRIN" IN THE PHOSPHATE-SALT BUFFER

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Abstract:

Objective: *The problem of secondary caries after restoration is actual in our days. Chlorhexidine is one of the most common antiseptics used in dentistry with proven bactericidal efficacy. The aim of this work was to predict long-term antibacterial activity, determine the presence and rate of diffusion of CHX from the "Restavrin" filling material into phosphate-buffered saline.****Materials and methods:*** *Samples of the composite material "Restavrin" were made with a concentration of 0.5% and 5.0% CHX. 4 ml of phosphate-buffered saline (PBS) with pH = 7.4 was added to polystyrene tubes. 10 tubes with PBS were prepared, in which were placed 4 disks of "Restavrin" filling material with 0.5% CHX, and 10 tubes with PBS were placed in 4 disks of Restavrin filling material with 5.0% CHX. All tubes were incubated for 70 days at 37 ° C.****Results:*** *The degree of sensitivity of microorganisms to samples of "Restavrin" filling material with 0.5% and 5.0% CHX when exposed to the PBS for 70 days is slightly reduced. No statistically significant differences were obtained when comparing the results of a study of the sensitivity of S. mutans and L. salivarius to samples of "Restavrin" filling material with 0.5% and 5.0% CHX and the results of a study of the timing of CHX diffusion in PBS.****Conclusion:*** *The prolonged presence of "Restavrin" filling material with AMA in the carious cavities, due to the weak solubility of CHX and its low diffusion into the oral fluid, has only a local effect and does not affect the composition of the oral biocenosis*

Keywords: *chlorhexidine, antiseptics, bactericidal efficacy, composite material, antimicrobial additives*

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INTRODUCTION:

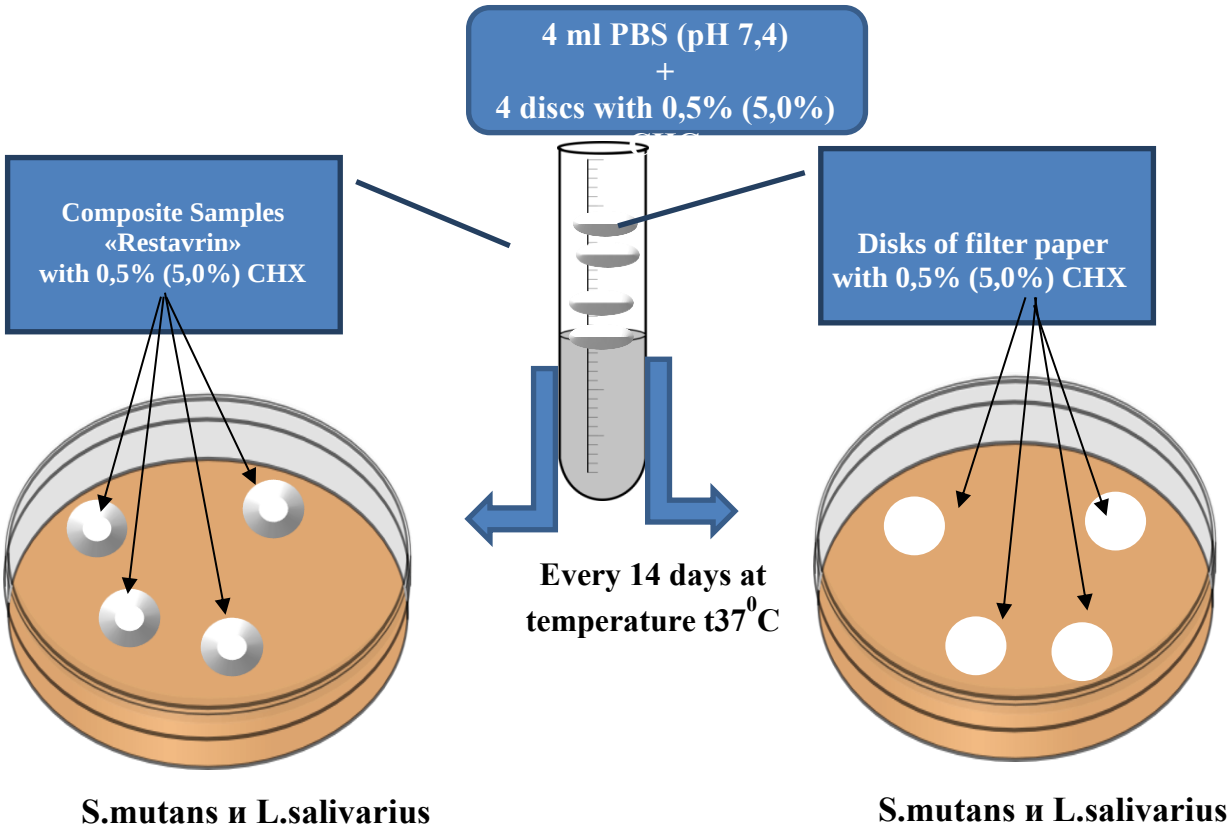
Chlorhexidine is one of the most common antiseptics used in dentistry with proven bactericidal efficacy. High activity against the main cariogenic microorganisms of the oral cavity provides prerequisites for the use of chlorhexidine to improve the methods and materials of treatment, as well as for the prevention of primary and secondary dental caries. Analysis of the literature indicates the need to improve the effectiveness of prevention and treatment of secondary caries, by improving and modifying composite filling materials, most often used in modern dentistry. In this regard, it is interesting and relevant to study the composite filling material with the addition of different concentrations of chlorhexidine acetate.

Aim: To predict long-term antibacterial activity, determine the presence and rate of diffusion (chlorhexidine acetate) of CHX from the "Restavrin" filling material into phosphate-buffered saline.

MATERIALS AND METHODS:

For research, samples of the composite material "Restavrin" were made with a concentration of 0.5% and 5.0% CHX by the method described above. 4 ml of phosphate-buffered saline (PBS) with pH = 7.4 was added to polystyrene tubes. 10 tubes with PBS were prepared, in which were placed 4 disks of "Restavrin" filling material with 0.5% CHX, and 10 tubes with PBS were placed in 4 disks of Restavrin filling material with 5.0% CHX. All tubes were incubated for 70 days at 37 ° C.

Every 14 days, 25 µl of the solution was taken from the tubes, soaked in filter paper discs, and placed on a Petri dish with inoculated test bacterial cultures. In the experiments, S. mutans and L. salivarius were used, the seeds of which were sown on the corresponding nutrient media (Table 4), and cultivated for 18-24 hours at a temperature of 37 ° C. At the same time, every 14 days, samples of filling material were removed from PBS tubes and placed on a Petri dish with S. mutans and L. salivarius plated. When bacterial growth appeared, the diameter of the zones of inhibition created around the disks of filter paper or samples of filling material was measured (Picture 1).

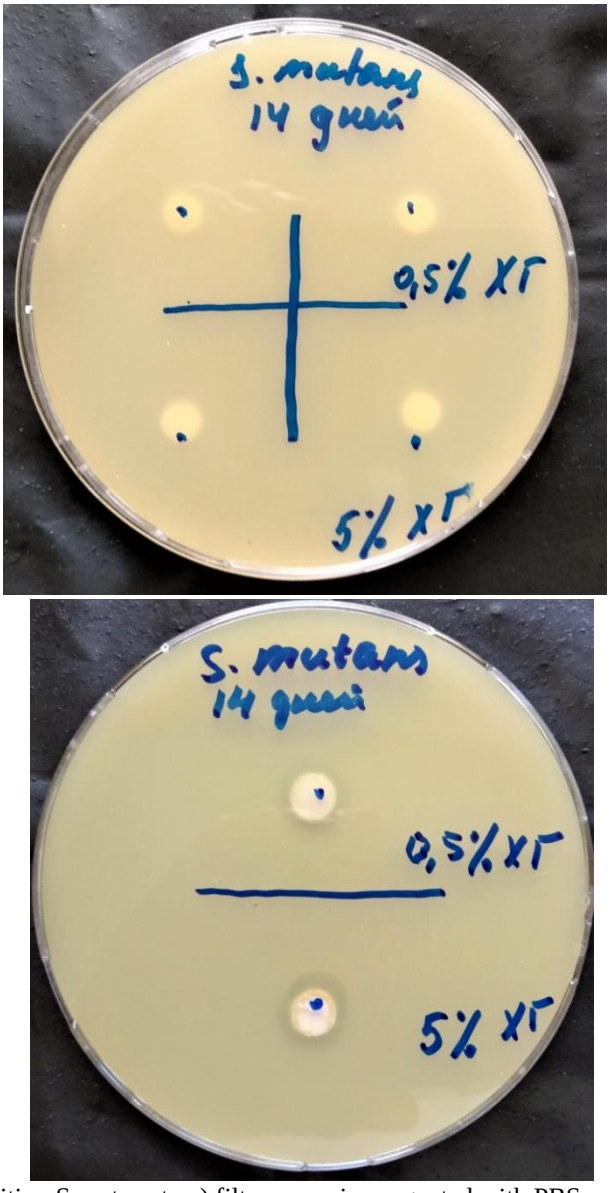


Picture 1. The study of the timing of diffusion of CHX from the filling material «Restavrin» in PBS pH 7,4

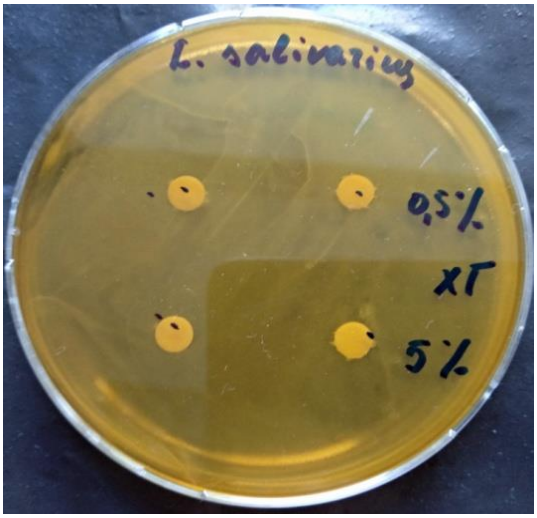
RESULTS:

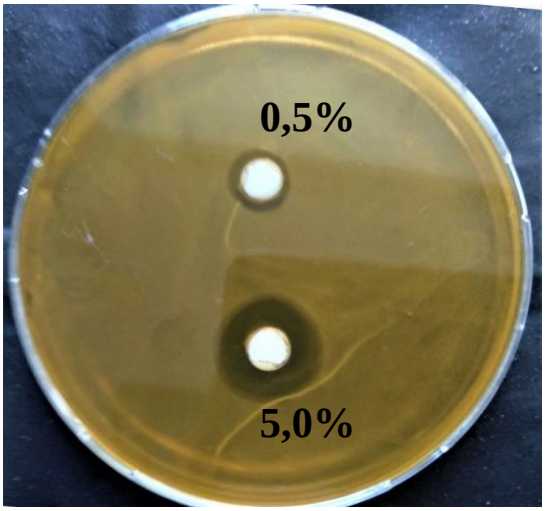
A logical question arises about the duration of the action of CHX, which is part of the studied composite filling material. To solve it, we conducted studies to study the diffusion of CHX and its activity during the long-term presence of samples of disks of filling material with antimicrobial additives (AMA) – chlorhexidine in a PBS solution at pH 7.4. Samples of "Restavrin" filling material with an antiseptic concentration of 0.5% and 5.0% were selected. Tubes with PBS and samples of the "Restavrin" composite material were incubated for 70 days at 37 ° C. Every 14 days, 25 µl of the solution was taken from two tubes, soaked in filter paper discs to study antimicrobial activity in relation to the clinical strains of S. mutans and L. salivarius. The results of the study after 14 days are presented in pictures 2, 3. At the same time, 4 samples of filling material were extracted from the same tubes, which were used to determine the preservation of antimicrobial activity for the same strains.

a) b)



Picture 2. Sensitivity definition S.mutans to a) filter paper impregnated with PBS solution, b) "Restavrin" filling samples with AMA concentration of 0.5% and 5.0% after 14 days of exposure in the PBS.

a) 

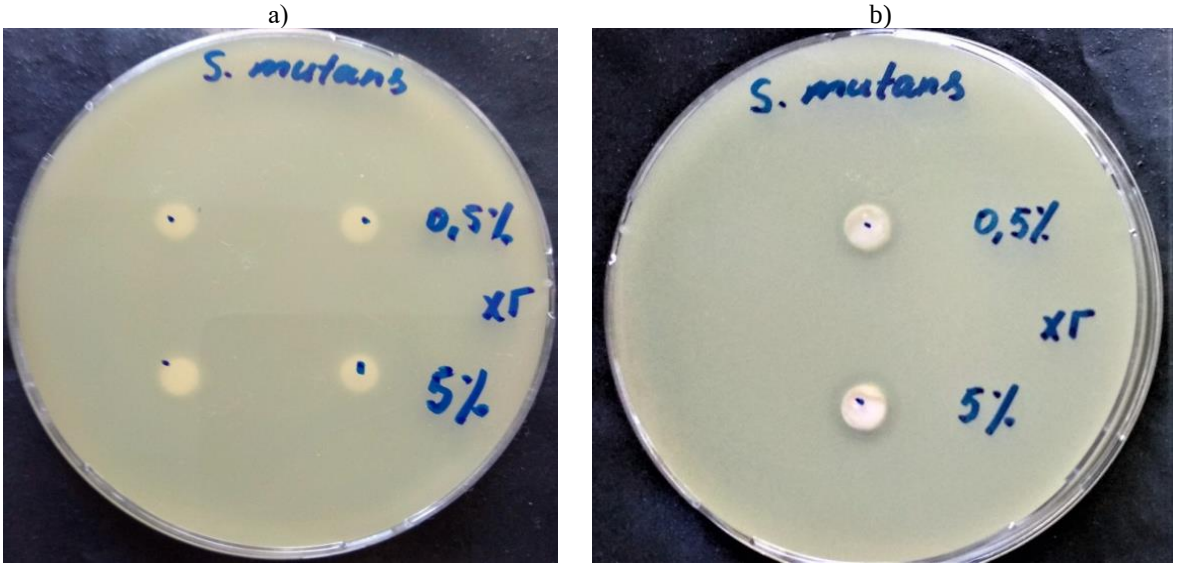
b) 

Picture 3. Sensitivity definition L. salivarius to a) filter paper impregnated with PBS solution, b) samples of Restavrin filling material with an AMA concentration of 0.5% and 5.0% after 14 days of exposure in the PBS.

After 14 days of exposure of the tubes with FSB at 37 ° C, it was not possible to detect the presence in the CHX solution by the method of paper discs. Filter paper disks impregnated with 25 µl PBS did not cause the formation of growth inhibition zones of S. mutans and L. salivarius on solid nutrient media in Petri dishes. The study of the samples of the "Restavrin" composite material with CHX itself, extracted from tubes with PBS after 14 days, showed that the bactericidal activity of the samples remained unchanged. For samples of a composite material with a CHX concentration of 0.5%, the S.mutans growth inhibition zone was 10.25 ± 0.29 mm, for 5.0% - 12.00 ± 0.47 mm. In L. salivarius, the growth inhibition zone for samples of a composite material with a CHX of 0.5% was 11.50 ± 0.33 mm, and for 5.0% it was 17.50 ± 0.33 mm.

After repeating the experiment after 28 days, filter paper discs saturated with 25 µl of FSB also did not cause the formation of growth inhibition zones of S. mutans and L. salivarius. For samples of composite material with a CHX concentration of 0.5%, the zone of S.mutans growth inhibition on solid nutrient media was 9.75 ± 0.29 mm, for 5.0% - 12.00 ± 0.47 mm (picture 4). Zones of growth inhibition of L. salivarius on solid nutrient media using Restavrin composite samples with CHX were almost similar to the previous ones and amounted to 11.25 ± 0.29 mm for samples with 0.5% AMA and 17.75 ± 0.55 mm for samples with 5.0% CHX (see table 1).

a) b)

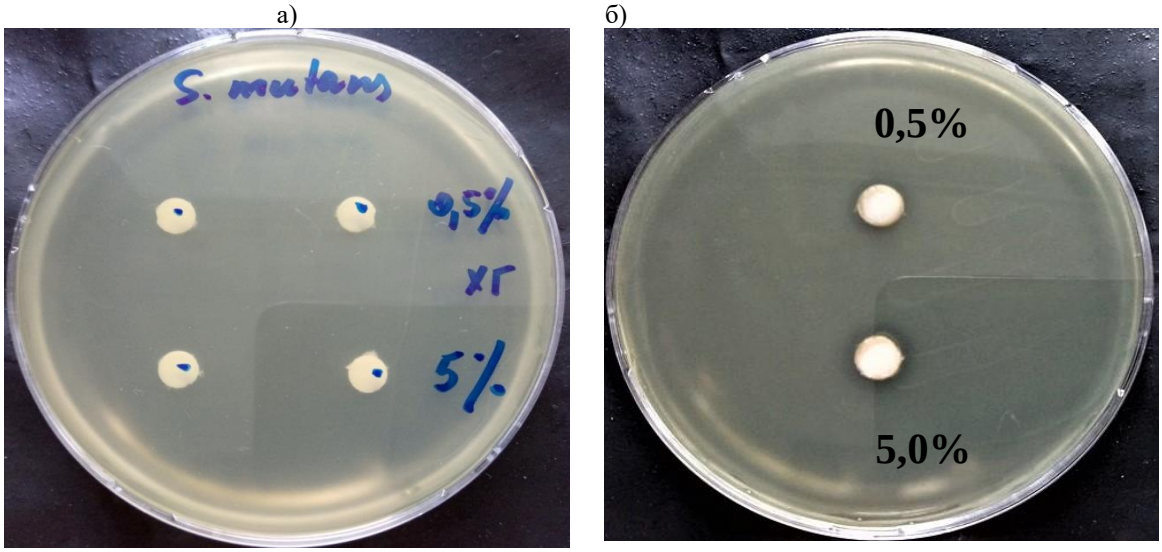


Picture 4. Sensitivity definition S.mutans to a) filter paper impregnated with PBS solution, b) samples of Restavrin filling material with an AMA concentration of 0.5% and 5.0% after 28 days of exposure in the PBS.

When the experiment was repeated after 42 and 56 days, growth inhibition of the studied microorganisms was also not revealed using filter paper discs saturated with 25 µl of PBS. After 42 days of exposure in the PBS, the S.mutans growth inhibition zone for samples of the "Restavrin" composite material with a concentration of 0.5% and 5.0% CHX was 10.50 ± 0.58 mm and 11.75 ± 0.55 mm. The growth inhibition zones of L.salivarius for samples of the composite material "Restavrin" with a concentration of CHX of 0.5% was 10.50 ± 0.33 mm, and 5.0% was 17.25 ± 0.29 mm. When the experiment was repeated after 56 days of exposure to the S.mutans growth inhibition zone for samples of the "Restavrin" composite material with a CHX concentration of 0.5% and 5.0%, no significant difference and amounted to 9.75 ± 0.29 mm and 12.00 ± 0,47 mm. The degree of sensitivity of L. salivarius to samples of the composite material "Restavrin" with a concentration of 0.5% CHX was 11.00 ± 0.47 mm, and with a CHX of 5.0% it was 16.50 ± 0.33 mm.

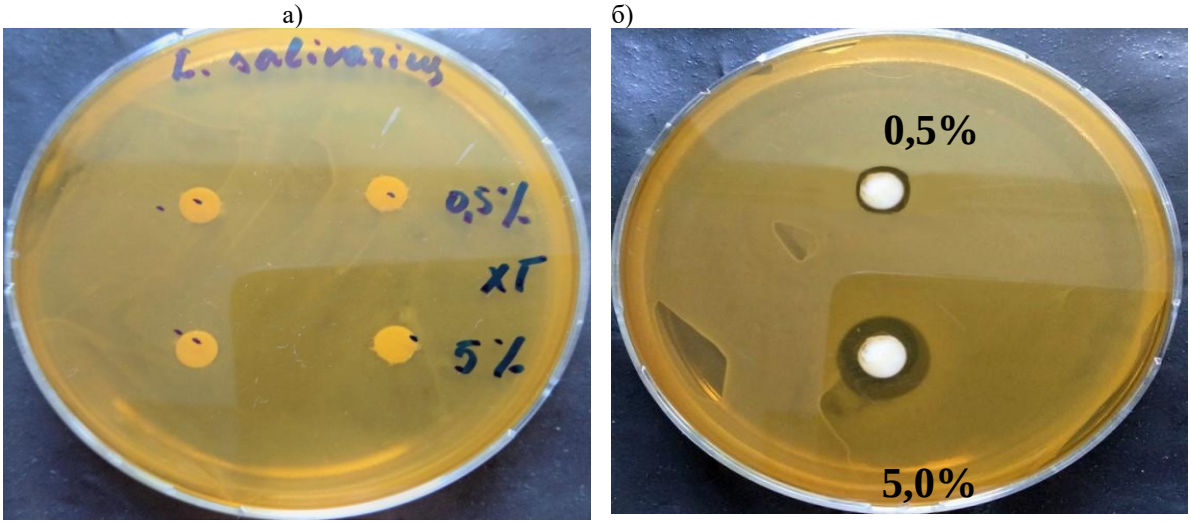
After 70 days of exposure of the "Restavrin" composite material to the PBS, it was not possible to detect the presence of CHX by using paper disks. Filter paper discs impregnated with 25 µl of PBS also did not cause growth inhibition zones of S. mutans and L. salivarius. The growth inhibition zone of S. mutans for samples of the Restavrin composite material with a concentration of 0.5% CHX was 10.00 ± 0.47 mm, for 5.0% - 11.25 ± 0.29 mm. The growth inhibition zone of S. mutans for samples of the Restavrin composite material with a concentration of 0.5% CHX was 10.00 ± 0.47 mm, for 5.0% - 11.25 ± 0.29 mm. (picture 5,6)

а) б)



Picture 5. Sensitivity definition S.mutans to a) filter paper impregnated with PBS solution, b) samples of Restavrin filling material with an AMA concentration of 0.5% and 5.0% after 70 days of exposure in the PBS.

а) б)



Picture 6. Sensitivity definition S.mutans to a) filter paper impregnated with PBS solution, b) samples of Restavrin filling material with an AMA concentration of 0.5% and 5.0% after 70 days of exposure in the PBS.

The generalized results of the study of the timing of diffusion of CHX in the PBS are presented in table 1.

Table 1. The sensitivity of clinical strains of S. mutans and L. salivarius to filter paper disks with 25 μl of PBS and "Restavrin" filling material with 0.5% and 5.0% CHX after exposure to PBS for 70 days

M/o	The duration of the exposure of the composite material "Restavrin" with the addition of CHX in PBS	Zone of inhibition in мм (M±m) at concentration CHG for filter paper discs with PBS		Zone of inhibition in мм (M±m) at concentration CHG for samples of composite material "Restavrin"		Student's criterion t , P
		0,5%* (n=4)	5,0%* (n=4)	0,5% (n=4)	5,0% (n=4)	
S.mutans	14 days	0	0	10,25±0,29	12,00±0,47	t=0,134,p>0,05**
	28 days	0	0	9,75±0,29	12,00±0,47	t=0,134,p>0,05***
	42 days	0	0	10,50±0,58	11,75±0,55	t=0,095, p>0,05****
	56 days	0	0	9,75±0,29	12,00±0,47	t=0,134,p>0,05*****
	70 days	0	0	10,00±0,47	11,25±0,29	t=0,015, p>0,05******
L.salivarius	14 days	0	0	11,50±0,33	17,50±0,33	t=0,026,p>0,05**
	28 days	0	0	11,25±0,29	17,75±0,55	t=0,069, p>0,05***
	42 days	0	0	10,50±0,33	17,25±0,29	t=0,017, p>0,05****
	56 days	0	0	11,00±0,47	16,50±0,33	t=0,005, p>0,05*****
	70 days	0	0	10,25±0,29	16,25±0,29	t=0,004, p>0,05******

Note: * 0-absence of a visible zone of growth inhibition corresponds to the diameter of the disk (d = 7); ** Student's criterion was determined by comparing growth retardation zones for composite samples with a CHX of 5.0% in the previous study and after exposure to the PBS for 14 days; *** Student's criterion was determined by comparing growth retardation zones for composite samples with a CHX of 5.0% in the previous study and after exposure to the PBS for 28 days; **** Student's test was determined by comparing growth retardation zones for samples of composite material with a CHX of 5.0% in the previous study and after exposure to the PBS for 42 days; Student's test was determined by comparing growth retardation zones for samples of composite material with a CHX of 5.0% in the previous study and after exposure to the PBS for 56 days; ****** Student's criterion was determined by comparing growth retardation zones for samples of composite material with a CHX of 5.0% in the previous study and after exposure to the PBS for 70 days.

An analysis of the results shows that the degree of sensitivity of microorganisms to samples of "Restavrin" filling material with 0.5% and 5.0% CHX when exposed to the PBS for 70 days is slightly reduced. No statistically significant differences were obtained when comparing the results of a study of the sensitivity of S. mutans and L. salivarius to samples of "Restavrin" filling material with 0.5% and 5.0% CHX and the results of a study of the timing of CHX diffusion in PBS. This may indicate that the diffusion of an antiseptic from the "Restavrin" composite material into the PBS is extremely slow and in small quantities**.** It should be borne in mind that CHX is a poorly soluble drug and is closely connected in the filling material, which will ensure its long-term effect on the microorganisms of the oral cavity locally, in the area of its location, and, therefore, will not have a bactericidal effect on the microflora of the entire oral cavity.

CONCLUSION:

An important aspect of the antimicrobial activity of "Restavrin" filling material for the treatment and prevention of caries is the duration of the action of CHX in its composition**.** To do this, we conducted a study of the timing of the diffusion of CHX from "Restavrin" filling material with an antiseptic in the

PBS. During 70 days, samples of "Restavrin" filling material with CHX at concentrations of 0.5% and 5.0% were exposed to PBS at pH 7.4. The results obtained indicate that the antimicrobial activity of the "Restavrin" composite samples from 0.5% and 5.0% CHX decreased slightly after 70 days of exposure in tubes with PBS. To detect CHX released from samples of a composite material in a PBS solution, filter paper impregnated discs were used. They did not cause the formation of growth inhibition zones of S. mutans and L.salivarius. These results can be explained by the fact that the diffusion of CHX from a composite material occurs slowly and in small quantities. At the same time, the activity of the "Restavrin" composite samples themselves remained almost unchanged (see table 1).

According to the literature, the study of the release of AMA from the composition of the filling material "Fuji IX" using high performance liquid chromatography showed that the introduction of crystalline chlorhexidine diacetate into the composition of the filling material at any concentration (from 1% to 3%) was not accompanied by its active diffusion. The insertion of chlorhexidine diacetate in complex with dihydrochloride in the form of a solution caused an increase in the level of leaching of AMA from the filling material [1].

Chlorhexidine in crystalline form is a poorly soluble substance, which contributes to its longer stay in the composite material and can provide a prolonged bactericidal effect on the microorganisms of the oral cavity locally in the area of the border of the hard tissues of the tooth with the filling, with natural abrasion of the surface of the filling material under chewing load. Based on the foregoing, it can be concluded that the prolonged presence of "Restavrin" filling material with AMA in the carious cavities, due to the weak solubility of CHX and its low diffusion into the oral fluid, has only a local effect and does not affect the composition of the oral biocenosis. This circumstance can play an important role when using the "Restavrin" composite filling material for therapeutic and prophylactic purposes.

REFERENCES:

1) Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomers cements containing chlorhexidine for the ART approach. Dent Mater. 2006 Jul;22(7):647–652
2) Dong Luo, Saroash Shahid, Gleb B. Sukhorukov, Michael J. Cattel Synthesis of novel chlorhexidine spheres with controlled release from a UDMA–HEMA resin using ultrasound Dental Materials Volume 33, Issue 6, June 2017, Pages 713-722.
3) Ferracane JL. Resin composite — state of the art. Dental Materials, 2011; 27: 29–38.
4) Kleverlaan CJ, Feilzer AJ. Polymerization shrinkage and contraction stress of dental resin composites. Dental Materials, 2005, 21: 1150–7.
5) Brunthaler A, Konig F, Lucas T, Sperr W, Schedle A. Longevity of direct resin composite restorations in posterior teeth. Clin Oral Invest 2003; 7: 63–70.
6) J.F. Zhang, R. Wu, Y. Fan, S. Liao, Y. Wang, Z.T. Wen, and X. Xu Antibacterial Dental composites with Chlorhexidine and Mesoporous Silica J Dent Res 93 (12) 2014. Pages 1283-1289.
7) S. N. Razumova, L.L. Gapochkina, R. M. Bragunova and all. Estimation of antimicrobial additive influence on composite properties. Medicinskiy alfavit №4 (33(336) 2017. Pages 24-26.
8) Demineralization of enamel in relation to the fluoride release of materials / N. Glaspool [et al.] // Am J Dent. - 2001. - №14. - P. 8-12.
9) Residual monomers (TEGDMA and Bis-GMA) of a set visible-light-cured dental composite resine when immersed in water / K. Tanaka, M. Taira, H. Shintani, K. Wacasa// J. Oral Rehabil. - 1991. - Vol.18. - P. 353 – 362
10) Al-Hashimi, I. Characterization of in vivo salivary-derrived enamel pellicle / I. AlHashimi // Arch. Oral. Biol. - 1989. - Vol.34. - P.289-295.
11) Whittaker, C.J. Mechanisms of adhesion by oral bacteria / C.J. Whittaker, CM. Klier, P.E. Kolenbrander // Ann. Rev. Microbiol. -1996. - Vol. 50. -P. 513-552.
12) Gibbons, RJ. Adsorbed salivary acidic proline-rich proteins contribute to the adhesion of Streptococcus mutans JPB to apatitic surfaces / RJ. Gibbons, D.I. Hay // J. Dent. Res. -1989. - Vol. 68. - P. 1303-1307.