

Comparison of the disinfectant effects of Nanosil D2 and Korsolex extra solutions on thermoset acrylic resin contaminated with *Streptococcus mutans* and *Bacillus subtilis*

Tahereh Ghaffari,
Fahimeh Hamed Rad¹,
Tahereh Pirzadeh²,
Gahanbaksh Asgari²

Department of Prosthodontics, Faculty of Dentistry, Tabriz University of Medical Sciences, ²Department of Bacteriology and Virology, Tabriz University of Medical Sciences, Tabriz, ¹Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

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ABSTRACT

This study was conducted to compare the disinfectant effects of Nanosil D2 and Korsolex extra on thermoset acrylic resin contaminated with *Streptococcus mutans* and *Bacillus subtilis*. In this experimental study, 90 acrylic samples were made and sterilized. Two samples were cultured as a sterilization control in brain-heart infusion (BHI) and the rest of samples were divided into two groups. Samples of one group were placed in a bacterial suspension of *S. mutans* and the samples of another group were placed in a suspension containing *B. subtilis*. Each group was divided into two subgroups for immersion in Nanosil or Korsolex extra solutions. Seven samples were selected from each group at each of 30 min, 1 h, and 2 h and transferred to the BHI test tube, and their turbidity was evaluated after 24 h. SPSS 17 software was used to analyze the data, and the significance level of test was considered $P < 0.05$. At 1 h, *Bacillus* level of Nanosil D2 was significantly lower than that of Korsolex extra, and at all ½, 1, and 2 h, the level of *Streptococcus* in Nanosil D2 solution was significantly lower than that of Korsolex extra ($P < 0.05$). *Bacillus* and *Streptococcus* levels showed significant reduction in both solutions over time. The disinfecting power of Nanosil D2 is more than that of Korsolex extra.

Key words: *Bacillus subtilis*, Korsolex extra, Nanosil D2, *Streptococcus mutans*

INTRODUCTION

Due to the contact with tools, individuals, and places, denture prostheses are at the high risk of contamination. Hence, preventing cross-contamination and disinfecting them are crucial. Physical methods are not as effective as chemical cleansing in reducing the number of denture-contaminating

microorganisms.^[1,2] Denture immersion in an appropriate disinfectant for adequate time to disinfect or sterilize is an easy and effective way so that it has been stated that denture immersion in sodium hypochlorite for 5 min can destroy various microorganisms, including spore-forming bacteria and *Candida albicans*.^[3] A minimum of 15 min of immersion in sodium hypochlorite or glutaraldehyde has been proposed to destroy the AIDS virus and hepatitis.^[4] Different times have been proposed for denture disinfection depending on the type of bacterial contamination and the type of disinfectant.^[2,5,6]

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Address for correspondence:

Prof. Fahimeh Hamed Rad,
Department of Prosthodontics, Faculty of Dentistry,
Babol University of Medical Sciences, Babol, Iran.
E-mail: fahimeh.hamedirad@gmail.com

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Disinfectants available in the market are divided into low-level, high-level, and intermediate-level types depending on the power of the effect. Some these substances include phenolic, alcoholic, and chlorine compounds known as intermediate-level substances. These disinfectants are suitable for killing *Mycobacterium*, vegetative bacteria, and most of viruses and fungi. Glutaraldehyde, hydrogen peroxide (H₂O₂), formaldehyde, and peracetic acid are known as high-level substances, used to kill all microorganisms, but they do not kill a large number of bacterial spores. In addition, alcohols, povidone-iodine, cresols, and chlorhexidine 4% are considered as low-level disinfectants. These substances may often kill most of vegetative bacteria and some fungi and viruses over a period of time.^[7] Among the wide range of existing disinfectants, the selection of them with high efficiency and fewer side effects is crucial for dentists. Although the time of using these chemicals is effective in denture disinfecting, the time should be reduced to a minimum, since it has been shown that more time affects the mechanical properties and stability of denture color.^[8]

H₂O₂ is used mainly for disinfecting and sterilization. It also affects the bacteria, viruses, yeasts, and spores and available at concentrations of 3%–90%. Wide range of effect, rate of effect, and long-term effectiveness, prevention of microbial re-contamination, lack of harmful effects on humans and the environment, and lack of creating microbial resistance even in long-term uses have resulted in its superiority and differentiation compared to other disinfectants.^[9] The synergistic effect of silver H₂O₂ in Nanosil D2 destroys a wide range of microorganisms, including the most resistant forms of them such as spores and biofilms to the weakest of them such as the HIV and hepatitis. This property makes the Nanosil D2 unique. Nanosil D2, like glutaraldehyde, is considered among the high-level disinfectants and can destroy all microorganisms.^[10] Korsorex extra is aldehyde broad-spectrum disinfection solution used for heat-resistant and heat-sensitive tools. This solution has very high adaptive power with different materials. Thus, it can be used for disinfection of glass, ceramic, stainless steel, aluminum, plastic, and hard rubber (such as latex and silicon) and artificial materials such as acrylic.^[11]

Studies show that denture prostheses are contaminated with *Staphylococcus aureus*, *C. albicans* and *Streptococcus* alpha-hemolytic,^[8] *Streptococcus* alpha- and beta-hemolytic, *Klebsiella*, and *Pseudomonas* species,^[12] and *Candida* colonies.^[13] *Bacillus* is a Gram-positive, aerobic, and spore-forming bacterium spread everywhere. *Bacillus subtilis* has some resistance to disinfecting processes due to its spore-forming property. Streptococci include a major part of oral flora. These bacteria play major role in decay, oral infections, and infectious endocarditis.^[14] *Streptococcus mutans* is part of the natural microflora of the mouth, and it can be used as a contamination marker outside the oral cavity.^[15]

Given the different results of disinfectants with various concentrations and chemical compounds, as well as the probability of contamination of dentures with various types of microorganisms showing different resistance to chemical substances, and as no study was found to compare effects of disinfectants Nanosil and Korsorex extra on acrylic resin, this study was conducted to evaluate the disinfecting effect of Nanosil D2 on Acropars acryl contaminated with *S. mutans* and *B. subtilis* and compare it with Korsorex extra disinfectant, assuming that the disinfecting effect of them is the same.

METHODOLOGY

This descriptive-analytical study was conducted in the Prosthodontic Department of Tabriz University of Medical Sciences. In this study, 90 smooth acrylic samples of Acropars 100 (Marlik Co, Eshtehard, Iran) were made in accordance with the ISO 1567 standard^[16] with a diameter of 17 mm and height of 6 mm. The specimens were sterilized by autoclave (Tecno-Gaz Evo Baganza-Parma, Italy) at 120°C with pressure of 1.4 bars for 15 min. Then, two samples as negative control were transferred to brain–heart infusion (BHI, E. Merck, 64271 Darmstadt, Germany), and after 24 h of incubation at 37°C, they were evaluated in terms of turbidity to ensure the accuracy of sterilization [Figure 1].

The remaining 88 samples were divided into two subgroups under the same conditions: One group was immersed in a microbial suspension containing *S. mutans* and another group was immersed in a suspension containing *B. subtilis*, prepared by the Iranian Pasteur Institute, which had a bacterial level equivalent to the half MacFarland. After 5 min, the samples were removed from the suspension and washed with sterilized distilled water.^[6] Then, they were placed on a sterilized dry gas. To ensure that the samples were contaminated, two samples of each group as positive control were transferred to the BHI medium, and 24 h after incubation at 37°C, they were examined in terms of turbidity. Then, each group was divided again to two subgroups (each contained 21 samples) and one group was immersed in a container containing Nanosil D2, (KimiaFam

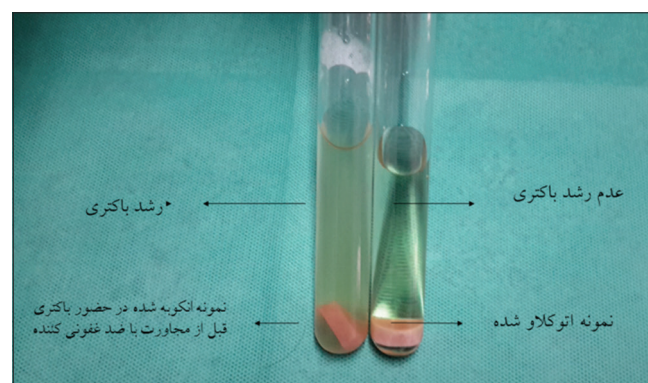


Figure 1: Examining the opacity of negative control samples

Table 1: Mean number of colonies produced in culture media after placing the samples in disinfectant solutions

Solution	Time	Bacillus			Streptococcus		
		Mean (CFU/ml)	SD	P ^a	Mean (CFU/ml)	SD	P ^a
Nanosil D2	0.5 h	933.33	103.28	0.645	183.33	58.20	0.000
Korsolex extra		900.00	115.47		950.00	100.00	
Nanosil D2	1 h	185.00	26.65	0.000	95.00	10.49	0.000
Korsolex extra		800.00	109.54		175.00	33.32	
Nanosil D2	2 h	91.67	28.58	0.502	18.00	4.77	0.000
Korsolex extra		103.33	29.44		88.33	20.41	
P ^b		0.000			0.000		
P ^c		0.000			0.000		

^aP: Mann-Whitney test to compare two solutions, ^bP: Kruskal-Wallis test to compare Nanosil D2 at different times, ^cP: Kruskal-Wallis test to compare Korsolex extra at different times. SD: Standard deviation, CFU: Colony-forming unit

Co, Tehran, Iran) and another group was immersed in a container containing Korsolex extra (Korsolex extra, BODE Chemie GmbH, Hartmann Co, Hamburg, Germany) and the container lid was closed.

At each time of 30 min, 1 h, and 2 h, seven samples were taken from each group and each sample was transferred to a test tube containing BHI, and after 24 h, colonies were counted [Figure 2].

The obtained information was recorded in the designed table and analyzed by SPSS 21 software (IBM Corp., Armonk, NY). Data were analyzed using Mann-Whitney and Kruskal-Wallis statistical tests.

RESULTS

The autoclave control sample did not cause turbidity in the liquid medium.

Contamination control samples made the tube turbid after adjacency with microbes and washing.

Investigating the effect of two solutions on *B. subtilis* showed that there is no significant difference in *Bacillus* level ½- and 2-h time. However, at 1-h time, the *Bacillus* level of Nanosil D2 was significantly less than that of Korsolex extra ($P < 0.05$) [Table 1].

Investigating the effect of two solutions on *S. mutans* showed that in all ½-, 1-, and 2-h times, this bacterium level in Nanosil D2 solution was significantly lower than that of Korsolex extra ($P < 0.05$). The results also showed that *Bacillus* and *Streptococcus* had a significant decrease in both solutions over time [Table 1].

DISCUSSION

In this study, the effectiveness of two chemical disinfectants (Nanosil 0.1% and 1/0 Korsolex extra 0.1%) was evaluated based on the reduction in the number of

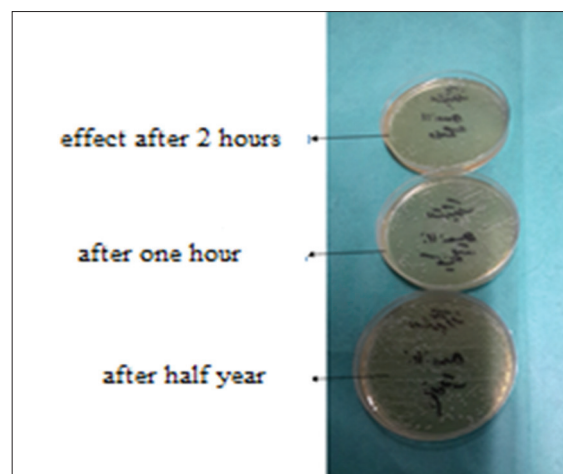


Figure 2: Effect of Korsolex extra on *Streptococcus mutans*

microbial colonies. The results of this study showed that there is significant relationship between the use of Nanosil and Korsolex extra solutions and reduction in the number of bacterial colonies of *S. mutans* and *B. subtilis* ($P < 0.05$). The greatest effect of Nanosil D2 solution was seen on *Bacillus* colonies in 1-h time, whereas the greatest effect was seen on reduction of *Streptococcus* colonies at ½ h. In this study, it was found that the bactericidal effect of D2 Nanosil was higher. The important point in this study is the effect of this solution on specific strains such as *B. subtilis*, which can cause stable contamination in an environment over time with producing spore. Ganavadiya *et al.* examined the disinfecting effectiveness of three chemical disinfectants (glutaraldehyde, H₂O₂, and ethyl alcohol) and concluded that the maximum reduction in the microbial load was achieved with H₂O₂ at first, and then, with glutaraldehyde. This result agrees with our research.^[17]

Badrian *et al.* evaluated the effect of three different disinfectants on alginate contaminated samples and concluded that Epimax (with H₂O₂ base) showed the highest reduction and it was effective in complete elimination of microorganisms in 10 min.^[18] Although these results are in line with the results of our study owing to superiority of

H₂O₂ in the complete elimination of microorganisms, they are consistent with those of our study due to not complete cleanup of microorganisms in our study. Differences in results might be due to the difference in disinfectants and their percentage, the microbes used, and the period.

In Nanosil D2, H₂O₂ prevents bacterial mass proliferation effective in periodontal disease due to antibacterial properties and oxygen releasing. Released oxygen destroys the protective membranes of the virus and the bacteria and enables the Nanosil to penetrate, the mechanism by which microorganisms are destroyed. Thus, Nanosil mouthwash acts better in anaerobic environment.^[19] Silver nanoparticles, compared to silver mass, create more contact surface and increase the antimicrobial effectiveness.^[20-23] The small size of silver particles in Nanosil increases its microbial properties by >99%.^[24,25] The antimicrobial property of silver ions is strongly dependent on covalent bonds to the bacterial proteins, leading to the deposition of proteins and the inactivation of the bacteria. Nanosil-coated products such as wound dressing, surgical instruments, skeletal prostheses, and contraceptive devices have been constructed. Moreover, researchers have referred to the possibility of using nano-silver canal disinfectants in endodontic treatments.^[26-29]

In a study conducted by Kangarlou *et al.* to compare the antibacterial effectiveness of a new canal cleaning solution containing nano-silver with that of sodium hypochlorite and chlorhexidine, it was shown that nano-silver solution has desirable antibacterial properties, and if its other properties are desirable, it could be used as a canal cleaning solution.^[30] Both of H₂O₂ and silver have synergic effects. The Nanosil manufacturer claims that it has no environmental damaging effects because the main components of this substance are water and oxygen, which are not toxic and contaminant.^[31] Nano-silver has killing effect not only on bacteria but also on a wide range of fungi, protozoa, and even viruses.^[32,33] As resin-base denture absorbs water and oral fluids, it can be thought that it can also absorb disinfection solutions which can later release into the mouth and cause allergic reactions.^[34] The high resistance of *B. subtilis* to the microwave has been reported, and this may be due to the spore-forming property of this bacillus. Bacterial spores are metabolically inactive and are particularly resistant to stressful conditions, such as heat and radiation. The exposure time should be longer than 2 min, in which destructive effects on the denture material will increase.^[35] Given the acryl longtime immersion in disinfectants in this study, further studies are needed to evaluate the destructive effects of these materials and to determine the minimum immersion time with maximum effectiveness.

CONCLUSION

In our study, the disinfecting power of Nanosil D2 solution at a concentration of 0.1% was higher than that of Korsolex extra.

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Conflicts of interest

There are no conflicts of interest.

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