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ORIGINAL ARTICLE

EVALUATION OF DISINFECTION METHODS FOR ANESTHETIC CARTRIDGES

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Abstract:

This study proposes a methodology to disinfect anesthetic cartridges used in the dental clinics of the Federal University of Amazonas. The research used 50 glass and 50 plastic cartridges. The anesthetic cartridges were disinfected through two different methods: immersion and friction. The experiments were carried out in two distinct sets: microbiological test and experimental validation, using 70% Alcohol, Polyvinylpyrrolidone, 2% Chlorhexidine, and 0.5% Chlorhexidine with Alcohol. The analysis of the results was based on mean and standard deviation statistical estimators obtained by the MATLAB software. Experiments conducted on plastic cartridges employing immersion in disinfectants elucidated that 2% Chlorhexidine and 0.5% Chlorhexidine produced better results than 70% Alcohol and Polyvinylpyrrolidone. For the experiments with glass material, 70% Alcohol and 2% Chlorhexidine presented the best results. In the experiments with different types of friction disinfections on the plastic material, the disinfectants 70% Alcohol and Polyvinylpyrrolidone presented similar performances. The disinfectants 2% Chlorhexidine and 0.5% Chlorhexidine obtained the best results compared to disinfectants 70% Alcohol and Polyvinylpyrrolidone. The analysis of experiments carried out with friction disinfection on glass found that the disinfectants 2% Chlorhexidine and 0.5% Chlorhexidine were similar. Moreover, the 2% Chlorhexidine and 0.5% Chlorhexidine disinfectants outperformed 70% Alcohol and 2% Chlorhexidine. Considering the efficacy of disinfection by disinfectant solutions evaluated in this study, the friction method with 2% Chlorhexidine proved to be more effective with the different disinfection methods.

Keywords: Dentistry, disinfection, anesthetics.

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INTRODUCTION

The materials used in the dental clinic cannot always be submitted to the sterilization process. Some special precautions that involve the degree of microbial contamination are necessary to control cross infection. For this reason, some disinfection measures must be used to minimize the risk of infection that may be present in the dental environment.

Disinfection is the process that eliminates microorganisms from objects and surfaces, except bacterial spores[1,2,3] and prions[4]. The Brazilian Ministry of Health, through ordinances, considers the mechanism of action and the specific use of chemicals for disinfecting surfaces and instruments, including products containing active principles such as quaternary ammonia; phenolic, iodized, and chlorinated compounds; iodophors and aldehydes[5].

Some products used in dental care, such as local anesthetic cartridges, have risk for cross infection in the dental clinic. The tube can be either plastic or glass, and both can serve as a shelter for bacteria if there is no appropriate disinfection method.

This work aims to compare the various methods of disinfecting dental cartridge solutions and the disinfection solutions used in the dentist's routine.

MATERIAL AND METHODS

The disinfection methods were carried out on cartridges containing solutions of local anesthetics, which are stored in the warehouse of the Faculty of Dentistry of the Federal University of Amazonas.

The number of cartridges used in this research was 100 anesthetic cartridges of 1.8 mL, packed in sealed blister packs. Fifty plastic cartridges: Lidostesim 2%(DLA Pharma, SP, Brazil) and fifty glass cartridges: Articaine 4% (DFL Indústria e Comércio S/A, RJ, Brazil). These cartridges were from different manufacturing batches.

Storage of boxes of anesthetic cartridges and disinfectant solutions evaluated was on the premises of the Microbiology Laboratory of the Faculty of Dentistry from the Federal University of Amazonas.

Disinfection of cartridges

In this research, the following commercially produced disinfectants were used: 70% alcohol (Audax Facilita, SP, Brazil), PVPI (Polyvinylpyrrolidone-Iodo – Rioquímica, SP, Brazil), 2% Chlorhexidine Digluconate (Rioquímica, SP, Brazil), 0.5% Chlorhexidine Digluconate in 70% alcoholic solution (Rioquímica, SP, Brazil).

Immediately after opening the packages, the cartridges were exposed for a period of seven days in the Microbiology Laboratory of the Federal University of Amazonas.

After this period, the examiner, using a pair of commercially sterile gloves (Medix Brasil, PR, Brazil), performed the initial collection to verify the contamination of the Control Group. A sterile swab soaked in saline (0.1 mL of 0.9% NaCl – ADV Farma, SP, Brazil), also sterile, was rubbed with continuous movement in a single direction against an anesthetic cartridge from each group, which will be detailed below. After collection, microbiological analyzes were performed in triplicate in Petri dishes with Brain Heart Infusion (BHI) culture medium (KASVI, SP, Brazil), and after incubation at 37 °C for 24 and 48 h. Then the growth of bacterial colonies was observed.

The disinfection of the tubes was performed by two different methods: friction and immersion. This step was necessary due to the previous exposure of the tubes in the Microbiology laboratory, causing them to be colonized by microorganisms and thus contaminated. Therefore, disinfection was necessary to assess the effectiveness of the disinfectants used in the present study. Eight microbiological tests were conducted, divided into four sets according to the packaging (plastic and glass), for a total of eight experiments in four different disinfectant products, namely:

a) Friction group (Figures 01 and 02): disinfection was performed, with the examiner wearing a pair of sterile gloves, and on plastic and glass anesthetic cartridges, secured with sterile clinical forceps. On an absorbent paper, the anesthetic cartridges were rubbed for thirty seconds with a gauze pad soaked with the disinfectant solution. Then, after the disinfection process, a sterile swab soaked in saline (0.1 mL of 0.9% NaCl), also sterile, was rubbed with continuous movement in a single direction against the cartridges. Immediately after collection, microbiological analyzes were performed in triplicate in Petri dishes with BHI culture medium, and after incubation at 37 °C for 24 and 48 h.

b) Immersion group (Figures 3 and 4): disinfection by immersion was performed, with the examiner wearing a pair of sterile gloves. The plastic and glass anesthetic cartridges, secured with sterile clinical forceps, were placed inside 15-ml Falcon centrifuge tubes (KASVI, SP, Brazil) containing 8 ml of the disinfectant solution. After sixty seconds, the anesthetic cartridges were left to dry on sterile absorbent paper. Then, a sterile swab soaked in saline (0.1 mL of 0.9% NaCl), also sterile, was rubbed with continuous movement in a single direction against each cartridge.

Immediately after collection, microbiological analyzes were performed in triplicate in Petri dishes with BHI culture medium, and after incubation at 37 °C for 24 and 48 h.

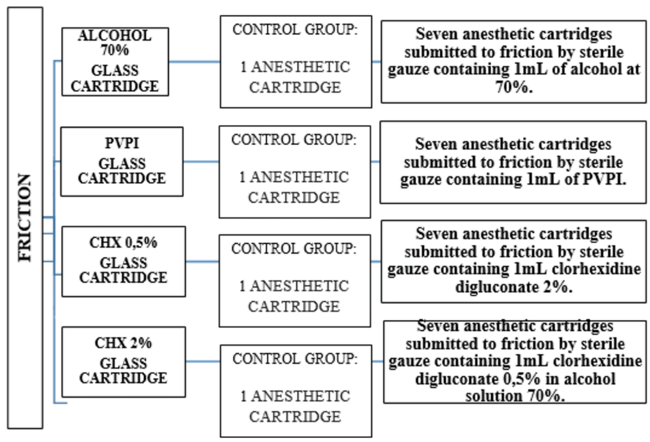

Figure 1. Demonstration of the four groups of glass cartridges submitted to friction disinfection.

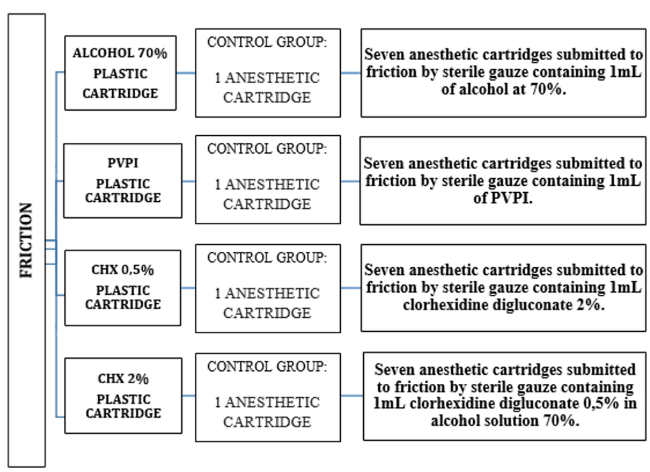

Figure 2. Demonstration of the four groups of plastic cartridges submitted to friction disinfection.

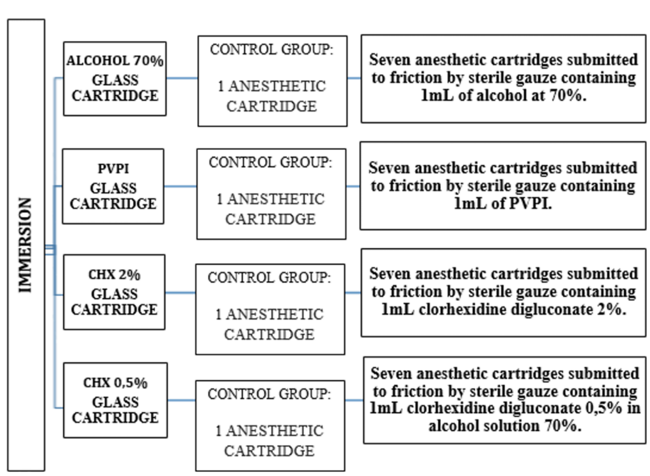

Figure 3. Demonstration of the four groups of glass cartridges submitted to immersion disinfection.

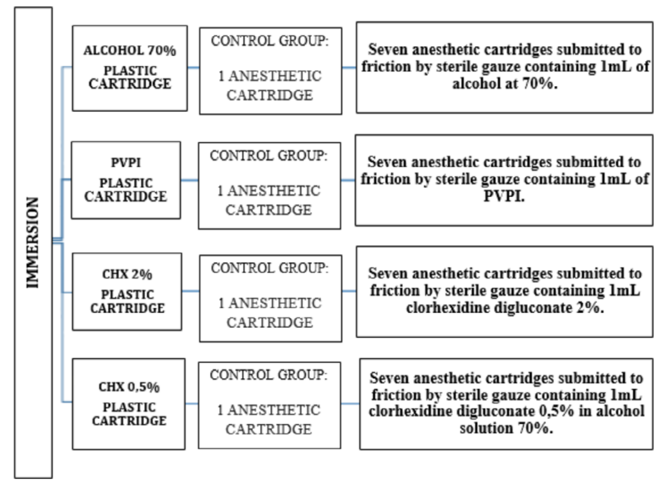

Figure 4. Demonstration of the four groups of plastic cartridges submeitted to immersion disinfection.

The procedures were renewed eight times, and after each experiment, the cleaning of the anesthetic cartridges was performed with water and neutral soap, and finally, submitted to sterilization.

Verification of disinfection

The verification of disinfection occurred at two different times: the first in 24 h and the second in 48 h. The dichotomous results of plaque evaluation were dishes without growth and dishes with growth. For this, laboratory equipment that facilitates digital counting of colonies of bacteria, fungi, and yeasts grown in Petri dishes, the Digital Colony Counter Model CP 600 (TECNAL, SP, Brazil), was used to view the growth of colonies.

Validation of the experiment

To validate the results obtained in the experimental phase, the experiments were repeated using the same conditions as the microbiological growth stage, but using a total of 2 experiments, maintaining 8 times for each experiment. To perform this step, another examiner performed the disinfection procedures (friction and immersion) on the glass and plastic cartridges in the same way.

RESULTS

For statistical analysis of this work, mean and standard deviation were calculated with the aid of the MATLAB software. The experiments were conducted in two different sets: experiments and validation. To analyze the results obtained, the criterion of $p \leq 0.05$ was used. Experimental results that are within the mean value with a tolerance of three standard deviations were included.

Each microbiological growth group tested, using each specific disinfection method and packaging material, was directly compared with the validation of the equivalent experiment.

Friction type disinfection and plastic material (Tables 01 and 02):

a) Although the 2% Chlorhexidine Digluconate disinfectant produced the best results for growth in 24 h and the alcoholic Chlorhexidine Digluconate showed better growth performance in 48 h, both disinfectants had slight growth variation in 24 and 48 h, and where superior to 70% Alcohol and PVPI disinfectants.

Friction type disinfection and glass material (Tables 03 and 04):

The 2% Chlorhexidine Digluconate and 0.5% Chlorhexidine Digluconate disinfectants exhibited the best performances, both for growth in 24 and 48 h, with a slight variation in 24 h and similar results in 48 h.

Immersion type disinfection of plastic material (Tables 05 and 06):

a) The 2% Chlorhexidine Digluconate and 0.5% Chlorhexidine Digluconate presented superior results to 70% Alcohol and PVPI for growth in 24 hours, with the last two results equivalent.
b) 0.5% Chlorhexidine Digluconate had better performance for growth in 48 h; however, the four materials obtained close performance for this type of growth.

Immersion type disinfection of glass material (Tables 07 and 08):

a) Alcoholic Chlorhexidine Digluconate achieved the best growth performance in 24 h.
b) 70% Alcohol and 2% Chlorhexidine Digluconate had the best growth results in 48.
c) The disinfectant 70% Alcohol showed superior performance to PVPI, both in growth in 24 h and 48 h.

Table 1. Preliminary results of the microbiological growth stage, carrying out seven experiments, with immersion disinfection and plastic cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			μ	σ	μ	σ
Immersion	Alcohol 70%	Plastic	5,14	1,34	0,28	0,49
Immersion	PVPI	Plastic	5,14	1,34	0,43	0,79
Immersion	CHX 2%	Plastic	0,14	0,43	0,38	0,53
Immersion	CHX 0,5%	Plastic	0,57	0,79	0,14	0,38

Table 2. Results of the validation phase of the results, using two experiments, with immersion disinfection and plastic cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2
Immersion	Alcohol 70%	Plastic	5	3	1	1
Immersion	PVPI	Plastic	4	2	2	2
Immersion	CHX 2%	Plastic	1	0	0	0
Immersion	CHX 0,5%	Plastic	0	0	3	1

Table 3. Preliminary results of the microbiological growth stage, carrying out seven experiments, with immersion disinfection and glass cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			μ	σ	μ	σ
Immersion	Alcohol 70%	Glass	4,86	1,57	0,43	0,53
Immersion	PVPI	Glass	5,29	2,06	0,71	0,95
Immersion	CHX 2%	Glass	0,86	0,90	0,57	0,96
Immersion	CHX 0,5%	Glass	0,57	0,77	1,00	1,00

Table 4. Results of the validation phase of the results, using two experiments, with immersion disinfection and glass cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2
Immersion	Alcohol 70%	Glass	3	4	2	1
Immersion	PVPI	Glass	4	1	2	3
Immersion	CHX 2%	Glass	1	0	0	0
Immersion	CHX 0,5%	Glass	1	0	1	0

Table 5. Preliminary results of the microbiological growth stage, carrying out seven experiments, with friction disinfection and plastic cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			μ	σ	μ	σ
Friction	Alcohol 70%	Plastic	2,00	1,00	1,71	0,49
Friction	PVPI	Plastic	1,29	0,76	2,00	1,00
Friction	CHX 2%	Plastic	0,00	0,00	0,29	0,49
Friction	CHX 0,5%	Plastic	0,57	0,53	0,14	0,38

Table 6. Results of the validation phase of the results, using two experiments, with friction disinfection and plastic cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2
Friction	Alcohol 70%	Plastic	3	1	0	0
Friction	PVPI	Plastic	4	1	2	0
Friction	CHX 2%	Plastic	0	0	2	0
Friction	CHX 0,5%	Plastic	0	0	3	0

Table 7. Preliminary results of the microbiological growth stage, carrying out seven experiments, with friction disinfection and glass cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			μ	σ	μ	σ
Friction	Alcohol 70%	Glass	2,57	1,61	0,57	0,53
Friction	PVPI	Glass	1,57	0,96	1,43	0,77
Friction	CHX 2%	Glass	0,28	0,76	0,14	0,38
Friction	CHX 0,5%	Glass	0,29	0,49	0,14	0,38

Table 8. Results of the validation phase of the results, using two experiments, with friction disinfection and glass cartridges.

Disinfection type	Disinfectants	Cartridges	Growth in 24 hours		Growth in 48 hours	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2
Friction	Alcohol 70%	Glass	4	1	1	0
Friction	PVPI	Glass	3	2	1	3
Friction	CHX 2%	Glass	0	0	0	1
Friction	CHX 0,5%	Glass	1	0	1	2

DISCUSSION

The concern with biosafety in dental care and the development of methods and protocols used to disinfect materials is important in the control of cross infections. Dentistry involves a constant risk of exposure to various environmental and human infectious agents that can affect staff, patients, and family members[6]. The current pandemic triggered by SARS-CoV-19 has led to a greater need to control infectious threats that could challenge these protocols, since dental materials can be contaminated by several pathogens after their use or even by their exposure to a contaminated clinical environment[7].

Anesthetic cartridges are commonly used in dentistry and, therefore, the number of microorganisms on their external surfaces needs to be reduced. A study by Bason et al.[8] found greater colonization of coconuts and gram-positive bacilli grown from anesthetic cartridges removed from the blisters a longer time than from cartridges removed from the blisters just before their use. However, Ranjbari et al.[9] demonstrated 6.3% contamination by aerobic cultures, 1.8% by anaerobic cultures, and 0.7% by fungal cultures, showing that the microbial contamination of the external surface of the cartridges is not negligible and even significant. Despite the study by Chutter[10] suggesting a specific method to sterilize these materials, the dentist's daily routine is to perform disinfection with different types of detergent solutions before their use.

In the scientific literature, disinfection protocols for anesthetic cartridges are scarce. Pauletti et al.[11] evaluated the effectiveness of disinfectants using the glass cartridge immersion method with analyzes of up to 24 hours. The present research helps to enrich the analysis of the disinfection process by including the friction method for both glass and plastic anesthetic cartridges, at times of 24 h and 48 h. Different disinfection solutions and techniques were tested, and the methodology used for their contamination was satisfactory because all groups of cartridges used in the research showed contamination and microbiological growth without the disinfection process.

We decided to use plastic and glass tubes because, although in many countries plastic cartridges are not available, they are still in use in Brazil.

All disinfectants chosen are recognized by ANVISA (Brazilian National Health Regulatory Agency) and showed proven efficacy, as corroborated in studies by Silva, Jorge[12] and Pauletti et. al.[11], who recommended their use. Among the disinfectants used, alcohol was the least effective, which was also observed by Payne[13]. The alcoholic Chlorhexidine solution showed the best results in reducing microorganisms, corroborating the findings of Silva, Jorge[12], and although we used different concentrations of Chlorhexidine (0.5%) from that author (5%), the product still obtained the best results.

Due to its low cost and low toxicity, and that it does not leave residues on dental equipment, 70% Alcohol is widely used in the dental clinic. Polyvinylpyrrolidone-Iodo (PVPI) has an excellent residual effect[1] and can also be used for pre-disinfection of anesthetic cartridges by friction[14]. In this study, the use of 70% Alcohol and PVPI presented similar performances in each disinfection method, but with differences in the period of microbiological growth. Because alcohol is chemically conditioned to its weight or volume concentration to water, when this relationship is unbalanced, its effectiveness is compromised, because when it enters the cell of the microorganism, it dehydrates without being able to kill it.

The disinfectants that contain Chlorhexidine Digluconate are more potent on microorganisms with increased concentration of this antimicrobial agent[14]. The alcoholic solution of 0.5% Chlorhexidine Digluconate is recommended as an immersion disinfectant[15]. The present research observed that the disinfectants 2% Chlorhexidine Digluconate and 0.5% Chlorhexidine Digluconate show similar results for both types of growth and techniques used, both on glass and plastic cartridges. However, the 2% Chlorhexidine Digluconate friction method was more effective in controlling cross infection. The choice of the friction method may be interesting because it uses less product than the immersion method, requires a shorter disinfection time, and has less risk of inadvertent entry of the product into the anesthetic cartridge, as is the case of small defects.

Therefore, anesthetic glass cartridges should be disinfected by rubbing for 30 seconds with 2% Chlorhexidine Digluconate. Immersion for 60 seconds in 0.5% Chlorhexidine Digluconate, 2% Chlorhexidine Digluconate, or 70% Alcohol are also options since all of these products performed similarly.

The disinfectants that showed the greatest effectiveness in the study contained 2% Chlorhexidine Digluconate, followed by 0.5% Chlorhexidine Digluconate in 70% alcoholic solution, 70% Alcohol, and PVPI, respectively, when considering the two methods of disinfection of anesthetic tubes – friction and immersion. However, when evaluated from the financial point of view, 70% Alcohol has the best financial cost, followed by 0.5% Chlorhexidine Digluconate in alcoholic solution, while 2% Chlorhexidine Digluconate and PVPI have similar costs.

CONCLUSION

Considering the effectiveness of disinfection by the studied disinfectant solutions, given that anesthetic tubes cannot be subjected to the physical sterilization process, like most materials used in clinical dental practice, the disinfectant solutions: 2% Chlorhexidine Digluconate and 0.5% Chlorhexidine Digluconate in 70% alcoholic solution presented superior results over 70% Alcohol and PVPI. The disinfectants Chlorhexidine Digluconate Alcohol, 70% Alcohol, and 2% Chlorhexidine Digluconate obtained the best results in the immersion method. For the friction method, 2% Chlorhexidine Digluconate is the most recommended for disinfection of anesthetic cartridges, whether plastic or glass cartridges. Despite having a higher financial cost, 2% Chlorhexidine Digluconate, when evaluated for its cost-effectiveness, remains the first choice of disinfectant and its use in the routine of dental clinics is recommended.

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