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Research Article

An in Vitro Effectiveness and Evaluation of Sodium Hypochlorite for Orthodontic Buccal Tube Disinfection

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Abstract

Contamination of fixed orthodontic components caused by non-compliance with hygiene both during the manufacturing and packaging processes can cause cross-infection. Although the literature has demonstrated the need for sterilization or disinfection of fixed orthodontic components prior to insertion in the oral cavity, these are still not widely used in orthodontic practice. This study aims to evaluate bacterial contamination *in vitro* and the disinfection effect of 0.5% sodium hypochlorite on orthodontic buccal tubes. A total of 12 roth type 1 Molar buccal tubes with slots of 0.020 and still well sealed in the packaging were divided into three groups: group 1 chlorhexidine 2% (n=4), group 2 distilled water (n=4) as control, and group 3 sodium hypochlorite 0.5% (n=4) as treatment. Microbiological and biochemical tests were carried out on the three groups of first molars to detect any bacterial contamination. The buccal tube showed that it was contaminated with bacteria and then disinfected using a 2% chlorhexidine solution, distilled water and 0.5% sodium hypochlorite. Bacterial contamination was detected in all control and treatment groups. The treatment group with 0.5% sodium hypochlorite disinfection showed optimal decontamination ($p > 0.05$) compared to the negative control group. Therefore, *In vitro* sodium hypochlorite 0.5% had the effect of reducing bacterial contamination of the buccal tube Roth type 1 molar with 0.020 slots.

Keywords: bacterial contamination; decontamination; sodium hypochlorite 0.5%

INTRODUCTION

The human oral cavity is a habitat for microorganisms that act as a reservoir for several pathogenic microorganisms that cause systemic infections and increase the risk of cross-contamination.[1] The use of fixed orthodontic components in the oral cavity can cause specific changes in the oral microflora by lowering pH, increasing dental plaque accumulation, and increasing the amount of microorganisms in saliva. These changes contribute to an increased risk of cross-contamination.[2] In addition, infections in the oral cavity can also be caused by the use of contaminated instruments or the direct use of orthodontic appliances received from the manufacturer's packaging without disinfection.[3]

Among the species identified by microbiological studies, *Streptococcus viridans* and *Staphylococcus spp*. are the most prevalent microorganisms found on the surfaces of dental equipment, which includes the methicillin-resistant S. aureus that has been detected on the surfaces of dental operatories, air-water syringes, and reclining chairs.[4] Almost all *Staphylococcus aureus* isolates produce the enzyme coagulase. This virulence factor

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also helps in finding sources. This organism is salt tolerant and able to grow on mannitol salt agar media containing 7.5% sodium chloride. *Staphylococcus aureus* in the liquid undergoes positive catalase and negative oxidase reactions.[5]

The buccal tube is a fixed orthodontic component in the form of a metal tube that is attached to the facial (buccal) surface of the tooth either on the orthodontic molar band or directly to the tooth surface, allowing the wire to pass when torsional forces are applied or allowing the wire to shift the tooth when movement occurs. The buccal tube, its surface area and the various joints of the hooks are potential areas for the attachment of bacteria, especially bacteria that cause periodontal disease.[6] Therefore, this buccal tube can be considered a semi-critical medical instrument that requires high-level disinfection.[7] *Staphylococcus aureus* is a facultative anaerobic bacterium, gram-positive, cocus-shaped and has the greatest pathogenic effect among the types of staphylococci in buccal tube orthodontics.[1]

Heat sterilization and disinfection are effective methods of removing microorganisms that cause contamination. However, the literature has reported that chemical disinfection is more effective in reducing contamination when compared to heat sterilization.[8] Disinfectants that are widely used and have disinfection effectiveness on pathogenic microorganisms are sodium hypochlorite. The use of sodium hypochlorite as a disinfectant is effective at a concentration of 0.5%.[9] Hypochlorite acid is a strong oxidizing agent, so it is capable of causing potential reactions with other molecules in oxidation-reduction reactions.[10] Chlorine can bind with components of the bacterial cytoplasm and form a composite N-cloro compound, which is toxic and can kill microorganisms.[11] These bacteria can grow 6-48 degrees Celsius in conditions of lack or absence of oxygen.[7]

The antimicrobial effectiveness of sodium hypochlorite, based on its high pH (action of hydroxyl ions), is similar to that of calcium hydroxide. The high pH of sodium hypochlorite disrupts the integrity of the cytoplasmic membrane by irreversible enzymatic inhibition, biosynthetic changes in cell metabolism and phospholipid degradation observed in lipidic peroxidation.[12] Based on the description above, the study aims to evaluate bacterial contamination of the orthodontic buccal tube and to assess the disinfectant effect of 0.5% sodium hypochlorite *in vitro*.

MATERIALS AND METHODS

This study utilized the True Experimental Post Test Only Control Group Design method, with a roth type 1 molar buccal tube with a slot of 0.022 as the research sample. The chlorhexidine group was a positive control, aquades was a negative control group, and the treatment group was treated with 0.5% sodium hypochlorite. In each group, 4 samples were needed based on the Slovin formula (n=N/ (1+N.e2). Research ethical clearance was obtained from the Health Research Ethics Commission, Faculty of Dentistry, Unissula No. 272/B.1-KEPK/SA-FKG/II/2021. The study began with a Buccal tube opened from a well-sealed factory packaging immersed in 3 ml of brain heart infusion (BHI) solution and placed in an incubator for 2x24 hours at 35^0C to evaluate the growth of Staphylococcus aureus.

The growth of *Staphylococcus aureus* was assessed based on the level of media turbidity with the Mc. Farland standard. Biochemical analysis was performed for tubes showing bacterial growth, and gram staining was performed and observed under a microscope to classify gram-positive and negative bacteria. *Staphylococcus aureus* is a spherical, gram-positive bacterium with a diameter of 0.7-1.2 μm, which is arranged in irregular clusters like grapes. Catalase test was performed to identify gram-positive isolates involved in orthodontic

buccal tube contamination. The buccal tubes in all treatment groups were disinfected with 0.5% sodium hypochlorite for 5 minutes and then dried for 6o seconds. Microbiological tests were carried out again on all buccal tubes to assess the efficiency of 0.5% Sodium hypochlorite solution.

RESULT

The average bacterial contamination from the spectrophotometric test results is shown in Table 1.

Table 1. Average Bacterial Contamination

Group	Number of samples	Mean	SD
Chlorhexidine	4	0.19	0.01
Aquades	4	0.87	0.07
Sodium Hipoklorit 0.5%	4	0.64	0.09

The highest *Staphylococcus aureus* contamination was in the distilled water group, followed by the 0.5% sodium hypochlorite group and the lowest in the 2% chlorhexidine group.

The results of the biochemical test are shown in table 2.

Table 2. Biochemical Test

Group	*Staphylococcus aureus*	Other Bacteria	Catalase test	Biochemical Test			
Chlorhexidine				Citrate	Urea	TSIA	MSA
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
Sodium Hipoklorit 0.5%							
1	-	+	-	-	+	-	-
2	-	+	-	-	+	-	-
3	-	+	-	-	+	-	-
4	-	+	-	-	+	-	-
Aquades							
1	+	-	+	-	+	-	+
2	-	-	-	-	-	-	-
3	+	-	+	-	+	-	+
4	-	-	-	-	-	-	-

The results of the biochemical test in Table 2 show that there is no bacterial contamination of *Staphylococcus aureus* in the 0.5% sodium hypochlorite and 2% chlorhexidine groups. However, there was still another coccus-shaped bacterial contamination that has not been specifically identified in the 0.5% sodium hypochlorite group. In the aqua dest group, there was contamination with *Staphylococcus aureus*. Shapiro-Wilk test in all groups P> 0.05 data showed that the data was normally distributed. Levene's test on the three groups p> 0.05 homogeneously distributed data. In One Way Anova test with p-value 0.000 (p>0.005), there was a significant difference in bacterial contamination of the orthodontic buccal tube between groups. Bonferroni's post hoc test demonstrated a significant difference between the 0.5% sodium hypochlorite group and the chlorhexidine group and not significant in the 0.5% sodium hypochlorite group with the distilled water group.

DISCUSSION

During the titration of sodium hypochlorite with a solvent, namely H2O, hypochlorous acid (HOCl) has been formed, which contained active chlorine. It

causes the bactericidal nature to be active. Chlorine compounds are strong oxidizing compounds. These compounds will oxidize the -SH group present in several essential enzymes so that it interferes with the metabolic function of bacterial cells.[13] Oxidation-reduction potential reactions caused by oxidation genes will produce new compounds such as hydrogen peroxide, superoxide, hydroxyl radicals and oxygen, most of which are mutagenic against bacteria.[14] In addition, protein denaturation occurs, leading to lipid oxidation in cell membranes and deactivation of enzymes causing damage to DNA.[15]

Furthermore, chlorhexidine is effective in reducing bacterial contamination. Chlorhexidine has a broad spectrum antibacterial activity, low toxicity and is soluble in water. This material is a strong base and is stable in the form of a salt. Chlorhexidine is a liquid antiseptic widely used as a chemical disinfectant. The exact mechanism by which chlorhexidine destroys bacteria remains unclear.[16] The existence of a bond or interaction between the positive charge of chlorhexidine and the negative charge of the phosphate particles of the bacterial wall allows the penetration of chlorhexidine into the cytoplasm of bacteria and causes a lysis effect. [16]The presence of permeable cell walls of gram-positive bacteria are easily destroyed compared to gram-negative bacteria. Therefore, higher chlorhexidine is required to kill gram-negative bacteria than is required to kill gram-positive bacteria.[17]

According to Omidkhoda (2017), chlorhexidine has a corrosive ability. Thus, when it comes into contact with metal materials such as buccal tubes, ions can be released. Thus, the use of 0.5% sodium hypochlorite can be utilized as an antibacterial and reduce the side effects of chlorhexidine.[18] Further *in vivo* studies are needed to support these findings. Disinfection before inserting the orthodontic buccal tube is very necessary because, based on research, there is still bacterial contamination when it is removed from the manufacturer's packaging.[19] The sold products of sodium hypochlorite with various levels of concentration can be directly used in various places. In Europe and North America, the chlorine concentration in products generally varies between 4% and 6%. [20]

CONCLUSION

Based on the research results, it can be concluded that 0.5% sodium hypochlorite had the effect of reducing bacterial contamination of the orthodontic buccal tube before insertion. Meanwhile, chlorhexidine had the highest effectiveness in reducing bacterial contamination of orthodontic buccal tubes before use.

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