Comparing the Effect of Chlorhexidine and Hydrogen Peroxide on Peri-implantitis Associated Strain of Staphylococcus aureus

Mansour Rismanchian¹, Saeid Nosouhian², Mohammad Shahabooei³, Farzaneh Nourbakhshian⁴

Contributors:
¹Associate Professor, Department of Prosthodontics, Dental Implants Research Center and School of Dentistry, Isfahan, University of Medical Sciences, Isfahan, Iran; ²Assistant Professor, Department of Prosthodontics, Dental Implants Research Center and School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran; ³Associate Professor, Department of Periodontology, Torabinejad Dental Research Center and School of Dentistry, Isfahan, University of Medical Sciences, Isfahan, Iran; ⁴Student, Dental Students Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.

Correspondence:
Nosouhian S, Hezar Jarib St, Dental Implants Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: +989131102651, Email: nosouhian@dent.uim.ac.ir

How to cite this article:

Abstract:
Background: Staphylococcus aureus is one of the critical microorganisms, which can cause peri-implantitis and endanger dental implants success rate. The aim of this study was to compare the disinfectant properties of chlorhexidine (CHX) and hydrogen peroxide (H₂O₂) on peri-implantitis associated strain of S. aureus.

Materials and Methods: Totally, 15 implant titanium disks were prepared in the same thickness and diameter. The disks were randomly divided into three groups (n = 5) based on the experimental disinfectants (CHX 0.12% and H₂O₂ 3%) and designated control groups. After the formation of a protein layer on disk surfaces, the specimens were exposed to S. aureus suspension. The decontamination procedure was completed during 5 min for both disinfectants. Trypsin protease 2% was applied to isolate the survived microorganisms at suspension of ½ and ¼. Muller Hinton agar culture was used for microbiota growth. After 48 h incubation, the standard colony forming unit was assayed. Finally, the collected data were analyzed by Kruskal–Wallis and Mann–Whitney tests using SPSS software version 22 at a significant level of 0.05.

Results: The Kruskal–Wallis test revealed the significant differences between study groups (P < 0.001). Furthermore, both groups presented significant differences with the control groups (all P < 0.01).

Conclusion: Both H₂O₂ and CHX are effective on S. aureus, nevertheless CHX seemed to be more lethal on studied bacteria but not significantly.

Key Words: Chlorhexidine, dental implants, hydrogen peroxide, periimplantitis, Staphylococcus aureus

Introduction
Nowadays, dental implants are proposed for replacing a missing tooth. However, the possibility of failure due to peri-implantitis (PI) is so concerning among clinicians. PI is an inflammatory response, which endangers implants and surrounding supportive tissues and brings about bone loss and implant failure finally.¹ The PI has a remarkable correlation with oral microflora and further immunological response. Therefore, the success of implant placement might highly depend on the colonization rates of microorganisms.² Many different microorganisms are involved in PI, but some of them seems to have more pathogenicity such as: Staphylococcus aureus (S. aureus), Staphylococcus epidermidis (S. epidermidis), Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, and Candida albicans (C. albicans).³⁴

In general, two methods are introduced to make significant decrease in bacterial biofilm on implant’s surface: Mechanical (such as using dental curettes, ultrasonic scalers, and nano abrasions) and chemical (such as using citric acid, hydrogen peroxide [H₂O₂], chlorhexidine [CHX], and antibiotics).⁵ Unfortunately, treatment protocol for PI remained uncertain but the decontamination of implant rough surface might be a hopeful solution.⁶ Periodontal debridement of pathogenic biofilms on the surface of titanium implants is somehow impossible, due to the design and texture of screwed shape implants. Hence, effective antiseptic therapy as a non-surgical procedure is recommended for PI.⁷ Bürgers et al.⁸ evaluated the antiseptic effects of six disinfectants on three PI associated microorganisms (S. epidermidis, Streptococcus sanguinis, C. albicans). The results manifested the significant effect of sodium hypochlorite (NaOCl) on the mentioned microorganisms.⁹ Another study evaluated the antiseptic effects of six antiseptic agents (NaOCl, H₂O₂ 3%, CHX 0.2%, plax, listerine, and citric acid 40%) on PI. The final results reflected the highest bactericidal effects of NaOCl, H₂O₂, CHX, and listerine.⁸

The aim of this study was to compare the antiseptic properties of the H₂O₂ and CHX on PI associated strain of S. aureus which was cultured on titanium implants.

Materials and Methods
This study was approved by the Research Ethics Committee of Torabinejad Research Center and Dental Implant Research
Center, Isfahan University of Medical Sciences, Protocol # 294028.

Sample preparation
In this analytical-observational in vitro study, 15 implant titanium disks (XiVE, Dentsply, Friadent GmbH, Mannheim, Germany) with the same thickness (2 mm) and diameter (4.5 mm) were prepared and randomly divided into three main groups based on the type of disinfectant and considering the control group.

The disks were sterilized by autoclaving (121°C for 15 min). To form a protein layer on the surfaces, the disks were stored in separate sterile eppendorf which were poured previously with 1.7 mL diluted horse serum (Biowest, Nuaille, France) by normal saline in 1:9 for 2 h at 37°C temperature.9

Contamination
After formation of the protein layer, the disks were transferred to S. aureus (ATCC 29213) microbial suspension (1.7 mL) and were remained for 60 h at 37°C temperature.

For many types of susceptible testing, standard inoculums of microorganisms must be used.

Hence, the standard inoculums were prepared according to 0.5 McFarland (1.5 × 10^8 colony forming unit [CFU]/ml) by transferring 1-2 colonies of 18-24 h culture stains to tryptic soy broth medium and incubating at 37°C.

In the next stage, the disks were expelled from the microbial suspensions and gently inserted in normal saline for three times to wash away any loosely attached microorganisms.9 Without any delay or drying, the decontamination procedure was performed on wet surfaces of the disks.

Decontamination
CHX 0.12% (Shahdaru Laboratories, Tehran, Iran) and H_2O_2 3% (Shimiran, Tehran, Iran) were used for decontamination, separately during 3 min.

Microbiological assay
After decontaminating, each disk was washed by 3 mL sterile distilled water for 30 s to omit any chemical remnants and avoid contamination or bias.

To isolate the survived microorganism as much as possible, trypsin protease 2% (AG Scientific Inc., CA, USA) was administered for 60 min. Then, the suspension of 1/2 and 1/4 Trypsin were prepared by using 100 µl samplers. These samples were transferred and spread on Muller Hinton agar culture media (Sigma-Aldrich, MO, USA) by using Pasteur pipet, which was heated to make a 90° bend. After 48 h incubating, the standard CFU was assayed, and the collected data were analyzed by Kruskal–Wallis and Mann–Whitney tests using SPSS software version 22 at a significant level of 0.05.

Results
The Kruskal–Wallis test revealed significant differences among all of the study groups (P < 0.001). Table 1 represents the mean CFU/ml values of different microorganisms after decontamination. Based on the result, the highest amount of remained bacteria belonged to H_2O_2 at a concentration of 1 (0.28 ± 0.62 × 10^3 CFU/ml). The mean CFUs were descending as their concentration descended in all the groups.

Table 2 manifests the pairwise comparison of different groups with the exclusive control group by Mann–Whitney statistical test. Based on the results, both of the groups exhibited significant differences with the control groups (all P < 0.01). However, there was no significant difference between both test groups (P = 0.07).

Discussion
S. aureus is one of the microbiotas of the human oral cavity, which can bind to hard surfaces.10 This bacteria can make pathogenic biofilms on various implant devises and has been turned to a major concern in dental implants failure.11 S. aureus is able to colonize on the titanium implant surface just 30 min after implantation and survive under different environmental conditions.12 Its pathogenicity is due to release of cytolitic toxins and virulence factors such as coagulase, catalase, and clumping factor A.13 Based on analyzed data, there was no significant difference between CHX and H_2O_2; however, the CHX presented better decontamination. CHX is a diphenyl compound with wide spectrum antibacterial activity on both Gram-positive and Gram-negative bacteria. It makes an alteration in the bacterial cell membrane and results in leakage and cell destruction.14 Furthermore, CHX inhibits glycosidic and proteolytic activities and reduces matrix metal-proteinase action in most oral bacteria.15 Carcuac et al. surveyed the

Table 1: The mean quantitate values (×10^3 CFU/ml) of remained bacteria after decontamination.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_2O_2</td>
<td>1</td>
<td>0.28±0.62</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>0.08±0.17</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>0.04±0.08</td>
</tr>
<tr>
<td>CHX</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5.85±0.00</td>
</tr>
</tbody>
</table>

S. aureus: Staphylococcus aureus, CFU: Colony forming unit, CHX: Chlorhexidine

Table 2: Pairwise comparison (P value) of different groups with the exclusive control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_2O_2</td>
<td>1</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>0.004</td>
</tr>
<tr>
<td>CHX</td>
<td>1</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>0.003</td>
</tr>
</tbody>
</table>

S. aureus: Staphylococcus aureus, CHX: Chlorhexidine
antiseptic effect of CHX on different titanium implants. Their result reflected minor treatment outcomes of CHX, which was differ based on implant surface characterizations. In the other hand, another study evaluated adjunctive effect of a dental water jet rinse mixed with CHX gel on PI. With respect to the technique, the flushing pressure of water jet might play a synergic role in decontamination of implant surfaces.

It has been reported that CHX disturbs streptococci attachment and its subsequent biofilm formation. Due to the fact that initial colonization of bacteria influences the later colonizers, CHX inhibits bacterial adherence and formation of biofilms.

In vitro experiments encounter some limitations, as they are observed in a static system compared to in vivo studies, which are more comprehensive due to various dynamic factors such as different systemic status, complex bacterial biofilms, and different immunological responses. Nevertheless, it might be considered that if the antimicrobial agent does not have activity in vitro it most likely will not work in vivo. Greater number of microbial species including indigenous and invaders species might reduce the accuracy of the antimicrobial testing. In the other hand, in vitro studies might provide a reference line for clinical studies.

Conclusion
Within the limitations of this in vitro study, it can be concluded that both H₂O₂ and CHX are effective on S. aureus, nevertheless CHX seemed to be more lethal on studied bacteria but not significantly.

References