Frequency of Contamination on Used Healing Abutments after Sterilization. An In Vitro Study

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Abstract:

Purpose: To determine and compare the frequency of contamination on different sites of healing abutments after sterilization with Phloxine B dye on unused and used healing abutments after sterilization. Materials & Methods: A total of 60 healing abutments were divided into two test groups: (1) used and sterilized and (2) a control group (unused). The test
group was evaluated for contamination after sterilization; the control group did not undergo any procedure. Data were analyzed using SPSS version 22. Descriptive statistics were used to determine the frequency of contamination in the different groups and at the different healing abutment sites. Chi-square test was used to evaluate the association of frequency of contamination with the site and design of the healing abutment. The level of significance was kept at \( P \leq .01 \). \textbf{Results:} The test group showed more contamination compared to the control. The most contaminated sites were the screw thread and the screwdriver engagement location. \textbf{Conclusion:} Reuse of healing abutments is cost-effective, but cleaning and sterilization was not effective for these components; thus, healing abutments need to be reused with caution as they were heavily contaminated when compared to new healing abutments. Among the different sites, the screw thread and screwdriver engagement sites were more prone to contamination. Healing abutment type did not influence the frequency of contamination. \textit{Int J Prosthodont} 2022. doi: 10.11607/ijp.7994

\textbf{Keywords:}
Cross infection; Decontamination; Phloxine B; Sterilization; Healing abutment

\textbf{Introduction:}
Dental implants are commonly used for replacement of missing teeth. Implant healing abutment guards the screw channel located inside the body of implant from the accumulation of debris during implant osseointegration. Reuse of healing abutments is a common practice, predominantly for financial reasons that can lead to cross-infection.

Infection control is extremely important in every field of medicine and dentistry to prevent transmission of infection.\textsuperscript{1} Guidelines suggest that every instrument that contact saliva and blood need to be sterilized after every dental procedure.\textsuperscript{2} Effective cleaning is a prerequisite to sterilization and it has been reported in the studies that cleaning of instrument is often overlooked and inadequately managed.\textsuperscript{3}

Dental implants are widely accepted treatment option for replacement of missing teeth, resulting in a significant improvement in chewing function and well-being of patients.\textsuperscript{4, 5} It penetrates the oral mucosa and form a transmucosal connection between inner parts of the body and the external oral environment.\textsuperscript{6} The implant healing abutment (HA) guards the screw channel located inside the body of implant from the accumulation of debris during implant osseointegration process.\textsuperscript{7}
Properly sterilized instruments and disease-free environment are essential for the placement of dental implant. Healing abutments are generally entitled for use only once as per manufacturer instruction in order to prevent cross-infection. However, for economic reasons many clinicians reuse it after cleaning and sterilization. Despite development of specific protocols, it is difficult to clean contaminated titanium surfaces effectively. Contamination of HA component arises from a variety of sources including: blood, saliva, food debris and epithelial cells. This may result in transmission of disease in cases where amino acids or protein are resistant to routine sterilization procedure. It also affects the screw friction when torqued to achieve the recommended preload at the time of final abutment placement.

Methods used to check contamination on used and sterilized HA include bacterial staining using dye and bacterial culture techniques. Phloxine B dye is a fluorescein derivative stain used as a protein and peptide highlighting stain. Bacteria stained with Phloxine B can be detected by eye without additional magnification and may be useful for staining both Gram-positive and Gram-negative bacteria.

Browne et al reported that titanium HA can be effectively sterilized. However Wadhwani et al. reported 90% of the HA screw driver engaging site was contaminated following cleaning and sterilization using dye method. Sahin et al. reported contamination in body (63.8%), screw driver hole (97.1%), screw (44.6%), occlusal (60.4%), and connector (40.6%) regions, respectively using the dye method. Cakan et al. reported contamination in 10.5% screw grooves and 5.2% driver slots on macroscopic observation and microbial growth in 5 of the 57 tested (8.7%) abutments. Due to highly debatable and controversial subject there was a need to plan research on this topic.

Therefore, the purpose of the study was to determine the contamination frequency around used but sterilized HA using a dye in order to evaluate the amount of residual contaminants and efficacy of sterilization procedure. The results might help us to identify the need to improve the sterilization protocol in order to minimize the chances of infection transfer among patients. These results will also help us in decision making regarding single use of healing abutments if they cannot be sterilized even after following the standard protocol of sterilization.

Materials and Methods:

An in-vitro experimental study was conducted at Dental Clinics, Sterilization department and tertiary care University Hospital, during January-March 2019. Non-probability consecutive sampling technique was used and a total of 60 healing abutments were included in the study. Our inclusion criteria was brand new and used healing abutment of two implant systems.
(Biohorizon and Zimmer) and exclusion criteria was damaged healing abutment (pitted, broken, corroded). The proposal of the study was sent to institutional Ethics Review Committee for exemption. Total of thirty used (test group) and thirty brand new (control group) implant HA meeting the inclusion criteria were collected from dental department of tertiary care hospital after taking informed consent from the dental department. We included the control group as negative control to confirm that the stain will only catch the contaminated area not the cleaned surface. For staining we used Phloxine B dye prepared by mixing 100µg powder and 1 ml deionized water. (Figure 1) The used healing abutment were collected immediately after the dental procedure. All used abutments were then cleaned and sterilized following the recommended steps including rinsing under running tap water immediately after use removing all blood, body fluids and tissue then placing the HA in ultrasonic bath containing distilled water for 10 minutes followed by placement in enzymatic solution, placed in sterilization pouches, sealed and coded with sample number and finally steam autoclave (121°C, 15 psi, 15-20 minutes).(Figure 2, A) After sterilization each healing abutment was removed from the sterilization pouch and placed in a micro-centrifuge tube containing 2 mL of Phloxine B (100µg/ml) dye (Figure 2, B) and was then placed in ultrasonic bath containing distilled water for 10 minutes. (Figure 2, C) After that each abutment was rinsed using deionized water and then will be air dried. A control group consisting of thirty brand new HA were also cleaned, sterilized and stained using the same procedure. Finally, the processed implant HA visualized under normal room light. (Figure 3) Photographs were taken using DSL camera (Canon EOS 70 D body and 1000 mm lens). All photographs were then thoroughly examined on a computer screen magnified at x15 by primary investigator for first time and repeated the evaluation for randomly selected half HA for second time to calculate intraexaminer reliability. For interexaminer relaibility another investigator repeat the evaluation process. Frequency of staining was recorded for each abutment for both the test group and the control group at the following four sites; 1) Screw driver engagement site 2) Body 3) Connection to implant fixture 4) Screw thread. The data collected was entered in data collection sheets.

Data Analysis:

SPSS version 22.0 was used for data analysis. Descriptive statistics was done to evaluate frequency and percentage of contamination on new and used healing abutment after sterilization. Chi-square/ Fischer’s exact test was applied to determine the frequency of contamination between the test and control groups. We stratified the data in order to control
the Effect modifiers like type and site of healing abutment. Post stratification Chi-square test was used. The level of significance was kept at $p$ value $\leq 0.01$.

**Results:**

Healing Abutments were divided into two groups (test and control), each group containing 30 healing abutments. Table I shows frequency of contamination after sterilization in the control and test group. There was statistically significant difference between the frequency of contamination between used (test) and unused (control). Test group (used and sterilized HA) showed more contamination as opposed to control (new and unused sterilized) HA. Table II shows comparison of contamination frequency between different sites (screw thread engagement site, body, connection, screw threads) of HA and type (Zimmer and Biohorizon) of HA. There was no statistically significant difference in frequency of contamination among different site and type of HA.

Table III shows comparison of contamination frequency after sterilization among different sites of healing abutment. There was statistically significant difference in frequency of contamination between screw driver engagement site and connection site and connection and screw thread site of healing abutment.

**Discussion:**

Reusing dental instrument is a common practice that brings a risk of cross infection if not properly clean and sterilized. Sources of contamination of HA include blood, saliva, epithelial remnants that are adherent to HA surface and are difficult to remove. Studies have reported that reusing healing abutment is beneficial both for patients and clinicians.$^{11-13}$ On the contrary many studies have reported against reuse due to risk of cross-infection.$^{7, 12, 14, 15}$ A study by Chew et al.$^{16}$ reported that cleaning of used HA is achievable with some extra caution and use of combination methods including autoclaving along with airpolishing and NaOCl.

Dye staining method was used in the present study for comparison of contamination between test and control group after sterilization. Phloxine B dye is a protein and peptide staining dye that can effectively stain the cellular debris and bacterial cells. Used HAs have been shown to contain different concentration of polysaccharides, proteins and cellular debris as part of biofilm biomass that can support the further adherence of disease triggering pathogenic organisms and identification of these contaminants is equally important to reduce the chances
of diseases transmission through used HAs.\textsuperscript{17-19} Therefore the present study focuses on using phloxine B dye that efficiently stains the cellular debris and help in the identification of residual contaminants on the used HAs.

Studies reported by Barreiros et al., Chew et al. and Cakan et al. have reported presence of viable pathogenic microorganisms and demonstrated that biofilms were not completely detached from the HA surfaces even after different cleaning and sterilization methods.\textsuperscript{8, 16, 20} Among the other methods used for evaluation of contamination, dye staining using Phloxine B was used, owing to its simplicity, cost effectiveness, ability to stain both gram positive and negative bacteria and ability to detect contamination visually by naked eye without any additional magnification.\textsuperscript{9, 10}

In the current study, used and sterilized HA (test Group) showed statistically significant contamination when compared to unused and sterilized (control group) HA. The frequency of contamination in the test group was 83.33\% as opposed to control group with the frequency of 6.66\%. Wadhwan et al.\textsuperscript{7} conducted a study to evaluate contamination in used and sterilized HA specifically protein using Phloxine B dye and reported contamination in atleast one site of 99 out of 100 HA that are similar to our study result. Sahin et al.\textsuperscript{10} also reported contamination in 99.4 \% of sterilized healing abutments received from different manufacturers. On the contrary study by Cakan et al.\textsuperscript{8} used both visual method and culture method to check contamination on healing abutment. They provided a contrasting result and concluded that 5 of 57 used and sterilized HA revealed microbial growth on culture media and 9 out of 57 showed dirty screw grooves and driver slots on macroscopic evaluation.

Browne et al.\textsuperscript{11} conducted a study to examine the contamination around sterilized used implant HA and impression and reported contrasting results and concluded that reuse of these components was safe as effective sterilization can be achieved after cleaning and steam autoclave and was comparable to new components in terms of sterility.\textsuperscript{11} Vezeau et al.\textsuperscript{21} conducted a research to check the ability of various cleaning and sterilization methods for the removal of biological debris. The author used simulated HA surfaces and contaminated them using serum for 1 hour to simulate oral environment and subjected to different sterilization techniques using steam autoclaving or ultraviolet light both with and without plasma cleaning. They concluded that plasma cleaning and sterilization using ultraviolet light can be effective for cleaning HAs, those using steam autoclave are questionable due to their tendency for organic and inorganic contamination and unfavorable surface alteration.
Contaminated surfaces not only poses a risk of cross infection it also effect implant in other ways like contaminated surfaces reduces the surface free energy that reduces the wettability and cellular attachment both epithelial and connective tissue. In the present study most frequently contamination type of HA (Zimmer versus Biohorizon) were also compared and found no statistically significant difference. A study by Wadhwani et al. demonstrated contradictory results to our finding and reported that healing abutments with circumferential grooves (Hiossen implants) in the design tend to exhibit more contamination due to difficulty in access the area during cleaning. He also reported that tapered HA has more contamination due to ledge design. The reason of contradictory results can be due to inclusion of healing abutments from multiple manufacturers having different design characteristics, however in the present study healing abutment of only two different manufacturers were evaluated.

The current study also assessed the most frequently contaminated sites and found statistically significant difference in contamination frequency among the different sites and types of healing abutments. Pair wise comparison demonstrated that screw driver engagement site and screw threads were the most frequently contaminated sites with the contamination frequency of 83.3% and 76% respectively, however the difference was not statistically significant (p-value ≤ 0.08). Similar results were also reported by Wadhwani et al. who also used Phloxine B dye staining method for assessment of contamination around different sites of HA and reported that total of 92 out of 99 screw thread site and 89 out of 99 screw driver engagement sites showed contamination. Chew et al. and Sahin et al. also reported similar results and found screw driver engagement site as the most highly contaminated area. The possible reason for more contamination in these sites were due to irregular grooved surfaces and difficulty in cleaning, hence sterilization was also affected. This not only poses a risk of infection, but these contaminated sites adversely affect the HA function.

If connection site is contaminated it will prevent accurate adaption at the level of implant abutment junction and allow more bacterial entry through the gap and ultimately affection the implant success. Repeated sterilization also cause scratches and damage to the HA surfaces specifically body of HA that provide a site for attachment of contaminants, become difficult to clean and cause peri-implantitis.

The strengths of the study were contamination of each site of a healing abutment of two implant systems was evaluated and the method used for detection of contamination was fast and cost effective. However, the limitations were that the study was conducted in only one center,
frequency of usage of HA was not taken into consideration, and we did not explore the nature of contamination on healing abutments.

We recommend multicenter study with large sample size and using different designs of healing abutment. Further work is necessary to develop tests that are more sensitive and specific like genomic testing and microbial culture for determining a quantifiable end point in the cleaning process. Appropriate education and training of CSSD dental staff in cleaning parameters should be emphasized. Instruments should be considered for single use only if cannot be cleaned and sterilized cannot be guaranteed.

**Conclusion:**

There was a significant difference in the frequency of contamination between new and used HA after sterilization. All the sites of healing abutment showed contamination but screw thread and screw driver engagement sites were the most frequently contaminated sites. Types of healing abutment did not have any significant difference in terms of overall contamination and among different sites of healing abutment. Reuse of healing abutment is cost effective but cleaning and sterilization was not effective for these components and need to be reused with caution.

**Funding information:** Self-funded. The authors report no conflicts of interest.

**References:**


Figure legends:

**Figure.1** Microcentrifuged tube containing Phloxine B Dye in powder form and falcon tube with prepared dye solution used to check contamination

![Microcentrifuged tube containing Phloxine B Dye and falcon tube](image)

**Figure. 2**

A. Sterilized Healing Abutments in Labeled & Sealed Pouches  
B. Sterilized Healing Abutments Placed in Labeled Microcentrifuge Tubes Containing Phloxine B Dye and Placed in Rack  
C. Sterilized Healing Abutments Placed in Ultrasonic Bath

![Sterilized Healing Abutments in pouches](image)  
![Sterilized Healing Abutments in microcentrifuge tubes](image)  
![Sterilized Healing Abutments in ultrasonic bath](image)
Figure 3

Stained (Contaminated) and non-Stained (Non-Contaminated) Healing Abutments
Table I: Comparison of Frequency of Contamination after Sterilization between Control and Test Group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Staining n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (New &amp; Sterilized)</td>
<td>1 (3.33)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Test (Used &amp; Sterilized)</td>
<td>25 (83.33)</td>
<td></td>
</tr>
</tbody>
</table>

- Chi-square test
- n = 60 (30/group).
- p-value ≤ 0.01
**Table II: Comparison of Frequency of Contamination among Different Sites of Healing Abutment and Type of Healing Abutment**

<table>
<thead>
<tr>
<th>Type of healing Abutment</th>
<th>Contaminated Healing Abutment Sites n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n =30 (15 sites/group)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Screw driver engaging site</td>
<td></td>
</tr>
<tr>
<td>Biohorizon</td>
<td>12 (80)</td>
<td>&gt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Body</td>
<td></td>
</tr>
<tr>
<td>Biohorizon</td>
<td>6 (40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Connection</td>
<td></td>
</tr>
<tr>
<td>Biohorizon</td>
<td>6 (40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Screw thread</td>
<td></td>
</tr>
<tr>
<td>Biohorizon</td>
<td>12 (80)</td>
<td></td>
</tr>
<tr>
<td>Zimmer</td>
<td>13 (86.66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body</td>
<td></td>
</tr>
<tr>
<td>Zimmer</td>
<td>11 (73.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Connection</td>
<td></td>
</tr>
<tr>
<td>Zimmer</td>
<td>5 (33.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Screw thread</td>
<td></td>
</tr>
<tr>
<td>Zimmer</td>
<td>11 (73.33)</td>
<td></td>
</tr>
</tbody>
</table>

- n=30(15 sites/group)
- Chi-square test / Fisher's Exact Test
- p-value <0.01 is significant
### Table III: Comparison of Frequency of Contamination after Sterilization among Different Sites of Healing Abutment

<table>
<thead>
<tr>
<th>Healing Abutment Sites</th>
<th>Staining</th>
<th>Screw driver engagement site VS Body</th>
<th>Screw driver engagement site VS connection</th>
<th>Screw driver engagement site VS screw threads</th>
<th>Body vs connection</th>
<th>Body versus screw threads</th>
<th>Connection Vs screw thread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screw driver engagement site</td>
<td>25 (83.33)</td>
<td>0.02</td>
<td>0.001</td>
<td>0.051</td>
<td>0.12</td>
<td>0.10</td>
<td>0.002</td>
</tr>
<tr>
<td>Body</td>
<td>17 (56.66)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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- $n = 120$ (30/site)
- Chi-square test
- $p$-value $\leq 0.01$

<table>
<thead>
<tr>
<th>Connection</th>
<th>11 (26.66)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Screw threads</td>
<td>23 (76.66)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>