International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Evaluation of Residual Oral Biofilm on Used Healing Abutments - An Invitro Study

Dr. Komal R Rayabagi[1], Prof (Dr.) Rakshith Hegde[2], Prof (Dr.) Pushparaja Shetty[3], Prof (Dr.) Chethan Hegde[4]

[1]Post Graduate Student, Department of Prosthodontics and Crown and Bridge, AB Shetty Memorial Institute of Dental Sciences, NITTE, Mangaluru, 575018, India
Email: raibagikomal[at]gmail.com
[2]Professor, Department of Prosthodontics and Crown and Bridge, AB Shetty Memorial Institute of Dental Sciences, NITTE, Mangaluru, 575018, India
Email: prosthodons[at]yahoo.co.in
[3]PhD, Professor, Department of Oral and Maxillofacial Pathology & Microbiology, AB Shetty Memorial Institute of Dental Sciences, NITTE, Mangaluru, 575018, India
Email: drpushparaj[at]nitte.edu.in
[4]Head of the Department and Professor, Department of Prosthodontics and Crown and Bridge, AB Shetty Memorial Institute of Dental Sciences, NITTE, Mangaluru, 575018, India
Email: drchethanhedge[at]nitte.edu.in

Corresponding Author

Prof (Dr.) Rakshith Hegde
[2]Professor, Department of Prosthodontics and Crown and Bridge, AB Shetty Memorial Institute of Dental Sciences, NITTE, Mangaluru, 575018, India
Email: prosthodons[at]yahoo.co.in

Abstract: ***Residual biofilm on reused healing abutments may compromise peri-implant health, making effective sterilization protocols essential. This study aimed to evaluate and compare the efficacy of two sterilization protocols in reducing protein-specific biofilm on healing abutments. A total of N = 40 healing abutments were analyzed, including 10 sterile pre-packed abutments (control, Group C) and 30 used abutments. The used abutments were divided into three groups: Group 1 (before sterilization), Group 2 (cleaning with enzymatic detergent and ultrasonic cleaning), and Group 3 (cleaning with enzymatic detergent, ultrasonic bath, and autoclave). All samples were stained with Phloxine-B, visualized under a stereomicroscope, and photographed. The percentage of surface staining was calculated to quantify protein-specific biofilm contamination, and data were compared using Fisher's exact test (P < 0.05). Significant differences in staining percentages were observed among the groups (P < 0.001). Group C showed no contamination (0% staining). In Group 1, high levels of biofilm were recorded with most samples showing 75% staining (N = 6) and one sample showing 100% staining. In Group 2, moderate contamination was observed with 50% staining in N = 6 and 75% staining in N = 4. In Group 3, the majority of samples showed 50% staining (N = 8) and the remainder 75% staining (N = 2). Both sterilization protocols reduced protein-specific biofilm compared to unsterilized abutments, but complete biofilm removal was not achieved.***

Keywords: biofilm, healing abutments, sterilization protocols, peri-implant health, protein contamination

1.Introduction

The successful outcome of implant supported prosthesis is largely dependent on the form and condition of the peri-implant environment [1]. Following dental implant placement, healing abutments (HA) are essential to the soft tissue healing process surrounding the implant. They provide support to soft tissues and facilitate their proper contouring around the implant fixture. They also act as physical barrier against bacterial colonisation at the implant-healing abutment component junction by promoting the adherence of epithelial tissue cells once they are implanted [2]. Although these components are intended for short- term usage, its duration in the mouth can range from a few weeks to several months, during which they remain in close contact with oral fluids and organic debris. This causes formation of strongly adhered biofilm on its surface [3].

It is widely advocated that these HAs are intended for single use, some clinicians and certain manufacturers have advocated the sterilization and reuse of HA [4]. This can cause infection due to transfer of biofilm containing microbial colonies from one person to another, ultimately compromising the peri-implant tissue health. In addition to this, repeated sterilization can alter the surface structure of abutments leading to detrimental effect on tissue due to bacterial contamination [5].

Various sterilization methods have been utilized to remove organic debris, biological contaminants (proteins and amino acids) and potential colony forming bacteria in biofilms. However, studies still point out that there will be some amount of residual matter and bacterial contamination after using these techniques [6].

Within the realm of dental implantology, the reuse of healing abutments has emerged as a topic of considerable interest and debate. Hence this study is an attempt to determine the efficacy of two different types of sterilization protocols on residual oral biofilm on healing abutments, which can prevent inflammatory response around the implant. The null hypothesis is, there is no significant difference in the protein

specific biofilm on healing abutments with varying sterilization protocols

2.Materials and Method

The study was approved by the institutional ethical committee. It is in accordance with the Helsinki Declaration of 1975 that provides for ethical practices. The dental practitioners were explained the purpose of the study and informed consent was obtained and the samples were collected. Sample size was calculated with 95% power and 5% alpha error and a total of 40 samples with 10 per group showed to be necessary.

The procedure was carried out in three steps.

Step 1- Collection of samples: In this in-vitro study 10 sterile healing abutments pre-packed from manufacturer (control group), 30 Healing abutments after being used for minimum of 6-8 weeks and not after being reused were collected from the private clinics.

Step 2- Sterilization: After collection, the healing abutments were initially inspected with unaided vision for presence of debris, damage or contamination. Thirty used healing abutments were randomly divided into 3 groups of 10 samples each. All the Samples were subjected to sterilization according to the group allotted. As a control, group c- sterile healing abutments pre-packed from manufacturer was used. In group-1, no sterilization was carried out with used abutment. In group- 2 used abutments were cleaned by immersing it in enzymatic detergent for 2-5mins (Rapid multi-enzyme cleaner) followed by ultrasonic cleaning at 40-45ºC for 10-15mins. In group- 3, cleaning of used abutment was done with enzymatic detergent for 2-5mins followed by ultrasonic bath at 40- 45ºC for 10-15 minutes and at last autoclave was done using Class-B autoclave at 134ºC for 12 minutes at 2.1 bar pressure.

Step 3: Protein biofilm analysis: All 40 samples were placed in individual plastic bag for staining with 2ml of Phloxine-B stain (Sigma Aldrich) and were sealed. Following which each healing abutments was rinsed in de-ionized water and allowed to air dry. The healing abutments were visualized with oblique light in stereomicroscope under 15X magnification and were photographed using digital camera (Nikon d3500 camera) at different sites. The protein specific biofilm which appeared as red stains were analysed and percentage of surface staining was calculated by one examiner by counting the number of stained surfaces visually, which indicates residual oral biofilm (Fig. 1).

Statistical analysis: The data was entered in Microsoft excel and analyzed using SPSS version 22. The data analysed was descriptive and inferential statistics. The descriptive statistics have been expressed in terms of number and percentage. Fisher exact test was used to compare the difference in protein specific biofilm among the 4 groups with $p<0.05$ was considered significant.

3.Results

The surface topography was analysed through the percentage of surface staining. In group C none of the abutments showed staining. In group 1 where in the staining experiment with phloxine B was used for healing abutments before sterilization, 3(30%) implants showed a 50% staining, 6 (60%) implants showed 75% staining and 1 (10%) implant had 100% staining. This difference was not statistically significant (p=0.178) (Table 1 and 2). In group 2 wherein the staining with phloxine B was used for healing abutments after cleaning with enzymatic detergent and ultrasonic bath, 6 (60%) implants showed a staining percentage of 50% and 4 (40%) implants showed a staining percentage of 75%. This difference was not statistically significant (p=0.527) (Table 1 and 3). In group 3 wherein the staining experiment with phloxine B was used for healing abutments after cleaning with enzymatic detergent, ultrasonic bath and autoclave, 8 (80%) implants showed a staining percentage of 25%, 2 (20%) implants showed a staining percentage of 50%. This difference was not statistically significant (p=0.058) (Table1 and 4).
A significant difference was observed in the staining percentage when all groups were compared (p=0.000) (Table 1) (Fig 2). On pair wise comparison of all groups to control group were significantly different (Table 5).

4.Discussion

The findings of our study suggest, group 2 and group 3 both reduced the surface biofilm, but were not able to completely eliminate them. The results are consistent with a study by Wadhwani et al. [10], who gathered 100 used and sterilized abutments and stained with protein specific stain. The results showed that Ninety-nine percent of the abutments showed protein contamination at one or more sites following cleaning and sterilization with ultrasonic bath between 10 and 60 minutes in various solutions and auto clave. Another study By Stacchi et al [12] gave similar result as in the current study which compared disinfection of HA with ultrasonic bath for 30 minutes with that of autoclave. They noticed that protein contamination was present in all (30/30) the HA treated with ultrasonic bath, were as 11 HA (11/30) in autoclave group which was statistically significant. Effectiveness of these two methods was extremely low as none were able to remove entire protein contamination which was also highlighted in the present study.

Limitations of the study: Evaluation of other non-protein or peptide residual contaminants and their source such as bacterial contaminants, host cell adherent material or food debris. An additional limitation was that only abutments made of titanium alloy were assessed, even though other alternative materials are also available such as stainless steel and zirconia. Categorization of abutments according to company could have been done as they vary with surface characteristics which have impact on residual contamination. Determination of percentage of contamination according to the surfaces could have made the results more accurate.

Cakan et al [6] studied the safety of using used healing abutments in clinical practice. They collected 60 sterile pouches from six implant dealers and tested their

sterilization. In accordance with the product catalogue and general sterilization guidelines, the implant companies autoclaved the utilized healing abutments that were taken from patients. After bacterial culture sensitivity and visual analysis by LED lamp, they concluded that sterilized abutments could not remove all contaminants. Results of this study are in contrast with our study i.e HA pre packed from manufacturer showed no contamination and was statistically significant. They suggested clinicians should clean and sterilize them again before use as a precaution. Barreiros et al [13] studied 85 abutments used for 3 months in oral cavity and stated that 30% of the abutments had remnant biofilm after cleaning followed by chemical disinfection with ultrasonic and autoclaving. The result in the present study showed that 8 out of 10 HA had surface contamination of 25% which was comparatively less than when compared to the study by Barreiros et al [13] and was significant. In addition, they also found that these remnant biofilms consisted of pathogenic species such as Aggregatibacter actinomycetem comitans, Prevotella intermedia, and Enterococcus faecalis which are found to cause periodontal diseases indirectly hinting the possibility of peri- implantitis.

A systematic review by Bidra et al [14] stated that that routine methods such as ultrasonic cleaning and autoclaving are insufficient in completely eliminating contamination on abutments. Nevertheless, no instances of adverse biological or mechanical effects or patient injury from the use of healing abutments have been found in the literature. Sánchez-Garcés et al [15] explored the eventual survival of microorganisms on sterilized healing abutments and to rule out the presence of transmissible organic material after standard procedures (cleaning with enzymatic detergent, ultrasonic bath and autoclave). They observed that none of the cultured abutments showed signs of bacterial growth, proving that the sterilisation was entirely effective in eliminating any living bacteria or spores. By analysing total organic carbon, they also ascertain whether any organic material is still present after sterilisation. Nevertheless, significant amounts of organic carbon may still be recovered after they have been sterilized. This is in terms with our findings which showed that phloxine b staining was present in all the used HA indicating incomplete removal of the organic material. Their study clearly showed that even though complete sterilization is obtained, organic matter remains to some extent as described in our results. Therefore, it is impossible to rule out the possibility of infectious particles like prions present on the reused healed abutments.

A survey by Paganotto et al [9], revealed that 0.07% of implantologists used ultrasonic cuvettes and enzymatic detergent as a method of eliminating organic matter. However, the removal of surface organic residues with this approach has been restricted. The results of Browne et al [16] states that impression copings and healing abutments when steam autoclaved and chemiclave showed sterility level equal to that of new one. However, there was no mention regarding removal of debris or any other contaminants. Result of this study contradicts our results as residual organic matter was more in chemical bath with ultrasonic than autoclave i.e. 50% staining was present in 4/10 and 2/10 respectively which was not significant. But, comparison these techniques with pre packed HA significant difference were noted.

Various studies have been carried out on other methods of sterilization in combination with autoclave, which have proved to be effective. Chew et al [7] used Phloxine B protein staining, scanning electron microscopy and light stereomicroscope concluded that highest area of biofilm was noted in autoclave alone sterilization. However, the combination of auto clave with air-flow polishing with erythrito and sodium hypochlorite contamination surface decreased. Naghsh et al [8] on comparing five decontamination approaches (autoclave, hypochlorite, chlorhexidine, air polish and hydrogen peroxide) reported that incorporating sodium hypochlorite and air polishing, with autoclaving, might serve as an efficient strategy to decrease residual contamination on the body surfaces of used titanium abutments. Kim et al [17] stated that steam sterilization is ineffective in customised abutments and chlorhexidine scrubbing and ultrasonic cleaning with chlorhexidine, acetone, ethyl alcohol is effective to remove bacteria and foreign bodies.

Mouhyi et al [18] performed a study on eight rats to evaluate the tissue response on contaminated and cleaned titanium screws in their abdomen. The cover screws were then cleaned by using citric acid, sterile water, hydrogen peroxide, and CO2 laser alone or with a combination of these. Results concluded that CO2 laser used alone or in combination with hydrogen peroxide may be used clinically for sufficient decontamination of titanium surfaces.

Future research should therefore investigate additional sterilization techniques beyond conventional ultrasonic cleaning and autoclaving, particularly combinations with air polishing, sodium hypochlorite, chlorhexidine, or CO_2 laser decontamination. Studies should also include evaluation of non-protein contaminants, as well as comparisons between abutment materials (titanium, stainless steel, zirconia) and different manufacturers, given their variable surface characteristics. Furthermore, detailed analysis of contamination on specific abutment surfaces may provide more accurate insights. Importantly, long-term clinical studies are needed to evaluate the biological impact of residual contamination on peri-implant health and to assess the potential risks associated with prion persistence.

5.Conclusion

This in vitro study concluded both the sterilization protocol could not completely remove the contamination on abutments. However, contamination can be reduced to certain extent by using enzymatic detergent followed by ultrasonic cleaning and autoclave. Considering this scenario, reuse of abutments has to be avoided until a standardized protocol or supporting evidence has been established to completely eliminate not only protein derived contaminants but other components as well.

Acknowledgement

The authors deny any possible conflict of interest related to this study.

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Tables

Table 1: Comparison of the staining percentage between all groups

Staining percentage	Groups				Total	p value
	Group 1	Group 2	Group 3	Control		
0%	0	0	0	10	10	0.000*
25%	0	6	8	0	14	
50%	3	4	2	0	9	
75%	6	0	0	0	6	
100%	1	0	0	0	1	

A statistically significant variation in staining percentages between the groups is shown in Table 1 ($p = 0.000$*). In all samples, the control group showed no staining, whereas Group 1 had the highest percentage of severe staining (75–100%). Different intermediate staining levels were shown by Groups 2 and 3. At $p < 0.05$, statistical significance is indicated by an asterisk (*).

Table 2: Staining before sterilization

Staining percentage	Observed N	Expected N	Residual	p value
50%	3	3.3	-.3	0.178
75%	6	3.3	2.7	
100%	1	3.3	-2.3	

The majority of samples have 75% staining, as indicated by the distribution of staining levels prior to sterilisation in Table 2. There was no statistically significant deviation from the expected distribution ($p = 0.178$).

Table 3: Staining after cleaning with enzymatic detergent and ultrasonic bath

Staining percentage	Observed N	Expected N	Residual	p value
25%	6	5.0	1.0	0.527
50%	4	5.0	-1.0	

Staining levels following enzymatic detergent and ultrasonic bath cleaning are displayed in Table 3. No significant deviation in the distribution ($p = 0.527$), and the majority of samples had 25% staining.

Table 4: Staining experiment with Phloxine B after cleaning with enzymatic detergent, ultrasonic bath and autoclave

Staining percentage	Observed N	Expected N	Residual	p value
25%	8	5.0	3.0	0.058
50%	2	5.0	-3.0	

Staining results following autoclaving, ultrasonic bathing, and enzymatic detergent cleaning are shown in Table 4. A greater proportion of samples displayed 25% staining.

Table 5: Pair wise comparison between groups

Sl No	Groups	p value
1	Group 1 v/s Group C	0.000*
2	Group 1 v/s Group 2	0.002*
3	Group 1 v/s Group 3	0.000*
4	Group 2 v/s Group C	0.000*
5	Group 2 v/s Group 3	0.314
6	Group 3 v/s Group C	0.000*

Staining level comparisons between groups are shown pairwise in Table 5. Most group pairs showed significant differences, especially between the experimental and control groups ($p < 0.05$). Nevertheless, there was no discernible difference between Groups 2 and 3 ($p = 0.314$). Statistical significance is indicated with an asterisk (*).

Figure Legends

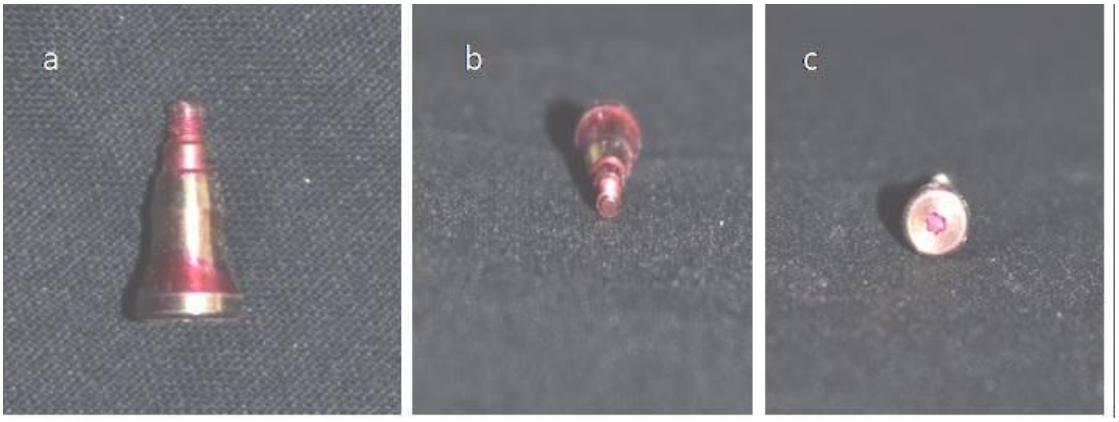

Figure 1: Protein and peptide stained with phloxidene B on healing abutment

Phloxine B-stained protein and peptide residues on a healed abutment are shown in Figure 1. Contamination of the abutment body (a), screw threads (b), and screw hole (c) is indicated by the red staining. These regions draw attention to biofilm retention zones that are frequently overlooked during insufficient cleaning.

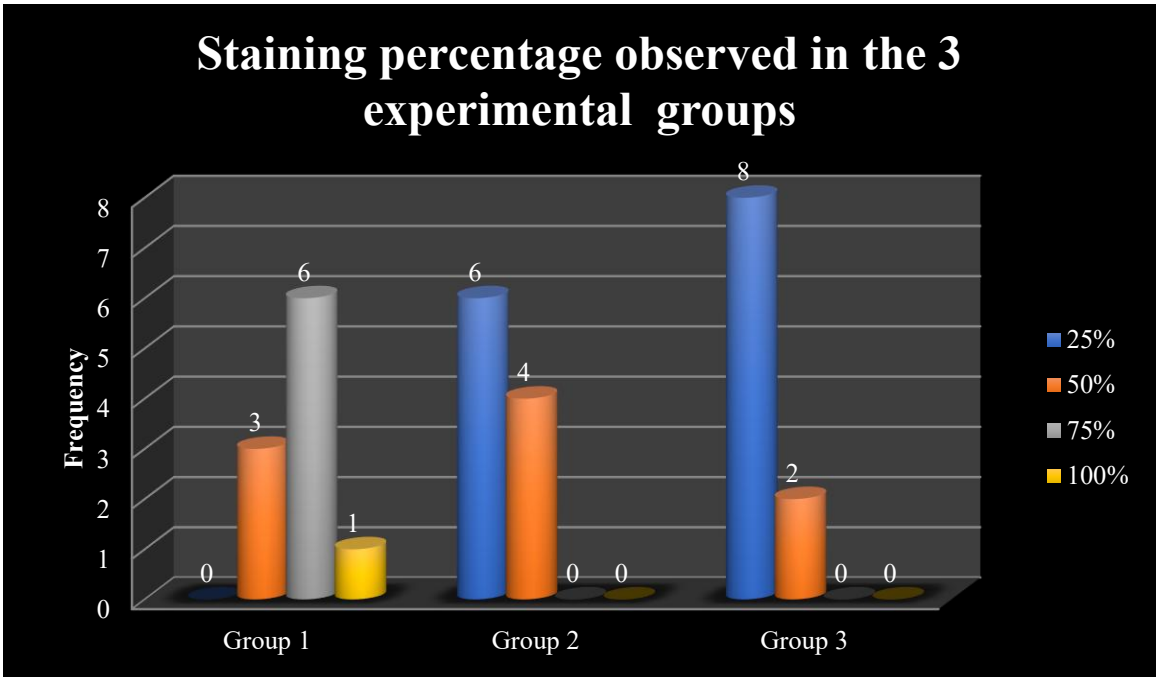

Figure 2: Comparison of the staining percentage between 3 experimental groups

The distribution of staining percentages among the three experimental groups is displayed in Figure 2. While Groups 2 and 3 displayed lower staining, with Group 3 having the highest frequency of 25% staining and no samples at 75% or 100%, Group 1 displayed higher staining levels, including 75% and 100%.