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Original Article

A comparison of four decontamination procedures in Reusing healing abutments: An in vitro study

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ABSTRACT

Objectives: This study aimed to compare the effect of four decontamination methods on the level of residual contaminants in the re-usage of dental healing abutments.
Materials and methods: In this experimental study, 50 used healing abutments were divided into five groups of ten as follows: 1. Control group: healing abutments were submerged in the ultrasonic device then autoclaved at 121 °C for 15 min; 2. Hypochlorite group: Same procedure as the control group, but the healing abutments were additionally immersed in 3 % hypochlorite for 20 min; 3. Chlorhexidine group: Same procedure as the control group, but the healing abutments were additionally treated with 12 % chlorhexidine; 4. Air polishing group: Same procedure as the control group, but the healing abutments were subjected to air polishing; 5. Hydrogen peroxide group: Same procedure as the control group, but the healing abutments were additionally exposed to 3 % hydrogen peroxide. Then, all healing abutments were stained with a protein-specific stain, Phloxine B. Five photographs were taken of each healing abutment, with four capturing the body (shank)and one capturing the top. All images were analysed, to measure the stained (contaminated) areas of each sample. The obtained data were analysed using statistical software (significance set at $p < 0.05$).
Results: The one-way ANOVA test indicated that the average percentage of contamination residues on the occlusal surface did not show a significant difference among the five groups: control: 5.5 ± 2.8, sodium hypochlorite: 4.9 ± 2.5, Chlorhexidine: 5.3 ± 2.5, air polisher: 3.1 ± 1.8 and Hydrogen peroxide: 4.8 ± 3.1. ($p = 0.26$). The average percentage of residual contamination on the body surfaces (shank part) was significantly lower in the air polisher (1.7 ± 1.1) and sodium hypochlorite (2.4 ± 1.1) groups compared to the other three groups (Control: 6.1 ± 2.3, Hydrogen peroxide: 4.6 ± 0.7, Chlorhexidine: 5.4 ± 2.4) ($p < 0.05$).
Conclusion: The results of this study showed that the use of sodium hypochlorite and air polishing, alongside autoclaving and ultrasonic cleaning, effectively reduced residual contamination on the body surfaces of healing abutments.

1. Introduction

The healing abutment (HA), usually made of titanium or a titanium alloy, serves as an intermediate, interim metal element used during the second step of implant surgery until the placement of a permanent prosthesis. During this time, it directly contacts soft tissues, facilitating the formation of a tight seal around the implant. This biological barrier prevents the penetration of bacteria and their products into deeper underlying areas, subsequently inhibiting infection of the pre-implant tissue, marginal bone loss (MBL), and further soft tissue recession. Additionally, it contributes to the development of an acceptable emergence profile in terms of aesthetics, particularly in the anterior region (Chokaree, Poovarodom, Chaijareenont, Yavirach, & Rungsiyakull, 2022; Odatsu et al., 2020).

One of the crucial requirements to achieve these objectives is to establish an aseptic environment, and a prerequisite for this is the utilization of sterile instruments. HAs are typically labeled as single-use or disposable items by companies. However, it is common for clinicians to reuse them for the same patient during prosthesis delivery or even for different patients (Kyaw, Hanawa, & Kasugai, 2020). Moreover, certain companies reprocess and resupply HAs after undergoing cleaning, sterilization, and repackaging for sale (Cakan, Delilbasi, Er, & Kivanc,

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2015). The underlying motivation for these practices primarily revolves around reducing expenses for both patients and clinicians, as well as minimizing material wastage in the industry (Kyaw, Abdou, Nakata, & Pimkhaokham, 2022b).However, concerns persist regarding the potential for cross-contamination and cross-infection, despite the implementation of conventional cleaning and sterilization methods (Bidra, Kejriwal, & Bhuse, 2020; Wadhwani, Schonnenbaum, Audia, & Chung, 2016). An inadequacy to eliminate the contaminations leads to a decrease in the adhesion, proliferation, and spread of fibroblast and epithelium cells in contact with the implant surface. Consequently, the formation of a robust biological barrier that prevents bacterial penetration is compromised, leading to the risk of pre-implant tissue infection and implant failure (Canullo et al., 2020).

As defined, sterilization refers to the complete removal of all viable microorganisms at the warranty level of acceptable sterility (Rees, 2012).However, research has indicated that removing protein and amino acid residues adhered to titanium surfaces can be challenging, and residual organic material may remain on HAs even after standard sterilization practices(Abreu, Estepa, Naqvi, Nares, & Narvekar, 2023; Almehmadi, 2021; Burioni et al., 2024; Gul, Zafar, Ghafoor, & Khan, 2024; Kyaw, Abdou, Nakata, & Pimkhaokham, 2022a).In a study by Wadhwani et al· (Wadhwani et al., 2016) the results revealed that 99% of HAs still had proteins and peptides remaining on one or more sites, even after following standard cleaning and sterilization practices. Moreover, a review (Bidra et al., 2020) concluded that conventional methods such as ultrasonic cleaning and autoclaving are insufficient in completely removing contamination for HA reapplication. On the other hand, investigations suggest that implementing a three-step protocol involving cleaning, disinfection, and sterilization could be a promising strategy to overcome these limitations (Gehrke et al., 2022).Some studies have suggested the application of air polishing with Glycine powder as a beneficial and safe method for removing residual contamination from HA (Cochis et al., 2013). Furthermore, the use of topical chemical disinfectant agents is common in dentistry, including sodium hypochlorite, chlorhexidine, and hydrogen peroxide.

Hydrogen peroxide offers several advantages over other chemical agents, including its broad spectrum of activity against various pathogens through the oxidation of diverse cell molecules. Furthermore, hydrogen peroxide is considered safe for use on open wounds and has been found to promote the proliferation of epithelial cells(Wiedmer, Petersen, Lönn-Stensrud, & Tiainen, 2017).Chlorhexidine, recognized as a gold-standard antibacterial agent, has demonstrated remarkable efficacy in inhibiting biofilm formation and gingivitis. It exhibits dose-dependent bacteriostatic and bactericidal effects. Moreover, it has demonstrated effectiveness against yeasts, dermatophytes, and certain lipophilic viruses (Bürgers, Witecy, Hahnel, & Gosau, 2012). Additionally, sodium hypochlorite, a well-established and traditional disinfectant widely used in dentistry for various applications such as endodontic treatment and reducing biofilm accumulation in removable prostheses, can be considered an excellent alternative as a disinfection agent. It offers beneficial properties, including bactericidal and fungicidal effects, tissue dissolving capabilities, and low toxicity at normal concentrations (Fukuzaki, 2006).

Based on the limited available evidence regarding the reuse of HA and the conflicting findings in research studies, our primary objective is to compare the efficacy of ultrasonic cleaning bath and autoclave, along with the utilization of air polishing, sodium hypochlorite, chlorhexidine, and hydrogen peroxide as additional options in a three-step method, in contrast to the conventional method. The goal is to assess the extent of residual contamination on the surface of healing abutments to determine their suitability for reuse. The null hypothesis in this study was that the amount of residual contamination in all groups and all surfaces was the same.

2. Materials and methods

This experimental study was conducted at the Dental Implants Research Center, School of Dentistry, Isfahan University of Medical sciences, Isfahan, Iran. The sample size for this study was calculated using the below formula, with an alpha level set at 0.05, an effect size (d=10%), and a power level set at 0.80, ($Z_{1-\frac{\alpha}{2}}$ =1.96, $Z_{1-\beta}$ = 0.84,and σ=16.7). A total of 50 HAs,10 per group showed to be necessary.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (\sigma_1^2 + \sigma_2^2)}{d^2}$$

Fifty healing abutments (UFII®, DIO Implant Co., Pusan, Korea) with a diameter of 4.5 and a height of 3 mm, which had been previously used once (for amount of at least 4 to 6 weeks in the patients' mouth) in fifty patients, were randomly divided by numerical draw into the following five groups, and each HA was assigned a code.

Group 1 (Control): 10 used HAs were subjected to a 10-minute submersion in the ultrasonic device, Eurosonic 4D (Euronda, Montecchio Precalcino (Vincenza) Italy) at 60 °C, followed by autoclaving (Melag, Euroklav 23V-S, Germany) in gentle program mode at 121 °C for 15 min (Wadhwani et al., 2016).

Group 2 (Hypochlorite): 10 used HAs were subjected to a 10-minute submersion in the ultrasonic device at 60 °C, followed by a 20-minute application of hypochlorite 3%. They were then washed with sterile saline solution for 1 min and autoclaved at 121 °C for 15 min (Gosau et al., 2010; Sonntag & Peters, 2007).

Group 3 (Chlorhexidine): 10 used HAs were subjected to a 10-minute submersion in the ultrasonic device at 60 °C, followed by a 5-minute application of chlorhexidine 12%. They were then washed with sterile saline solution for 1 min and autoclaved at 121 °C for 15 min (Mariotti & Rumpf, 1999).

Group 4 (Air polish): 10 used HAs were subjected to 10-minute submersion in the ultrasonic device at 60 °C, followed by air polishing system with glycine powder (Perio-mate, NSK, Japan) for 15 s at a distance of 5 mm from the surface and an inclined angle of 45–60 degrees. They were then autoclaved at 121 °C for 10 min (Chew, Tompkins, Tawse-Smith, Waddell, & Ma, 2018).

Group 5 (Hydrogen peroxide): 10 used HAs were subjected to a 10-minute submersion in the ultrasonic device at 60 °C, followed by a 1-minute application of hydrogen peroxide 3%. They were then washed with sterile saline solution for 1 min and autoclaved at 121 °C for 15 min (Alotaibi, Moran, Grufferty, Renvert, & Polyzois, 2019).

After the aforementioned procedures, all HAs were packed in sealed pockets containing 2 ml of Phloxine B solution (Sigma Aldrich). Phloxine B is a fluorescein derivative stain and one of its applications is to detect proteins. Proteins can get degraded to tenacious biological parts known as prions' which are infectious agents that can retain their infective potential over time. Recent studies have used Phloxine B to evaluate the ability of various cleaning protocols to clean dental implant abutments (Rasooly, 2005; Bali, Bali, & Nagrath, 2011; Stacchi, Berton, Porrelli, & Lombardi, 2018; Chew, Tompkins, Tawse-Smith, Waddell, & Ma, 2018).

. Subsequently, the HAs were placed in ultrasonic bath for 10 min and then rinsed in deionized water and dried at room temperature (Wadhwani et al., 2016) (Fig. 1). The HAs were then observed and imaged using a Stereomicroscope (Trinocular Zoom Stereo Microscope, SMP 200, HP, USA) equipped with a digital camera (Moticam 480 Digital camera, SP10.0224, Motic Instruments Inc., USA) at ×15 magnification. Five photographs were taken of each HA, four of the body surfaces and one of the top surfaces. To capture images of the surfaces, the HAs were secured within a rectangular putty mold with four equal surfaces (Figs. 2 and 3). The putty molds were rotated 90 degrees three times to obtain four images of the body surfaces and one image of the top

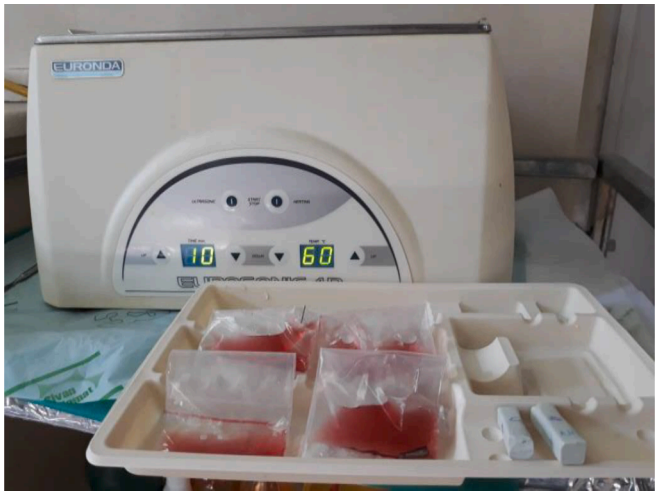

Fig. 1. Ultrasonic device and HAs placed in phloxine B solution.

Fig. 2. Stereomicroscope and a rectangular putty mold.

surface for each HA. Subsequently, all images were analysed using Cool PHP tools software (NJ, USA) with color code to measure the stained (contaminated) areas of each sample. The contamination surface area was expressed as a fraction (%) of the total surface area within the image pixels (Langbein, 2016).For Statistical Analyses, the obtained data were analysed using one-way ANOVA, and Tukey's post-hoc tests, utilizing the SPSS statistical software version 22.0. The significance level was set at $p < 0.05$.

3. Results

The analysis involved 50 HAs to identify the fluoxetine B-stained surfaces (contaminated surfaces). The results of the one-way ANOVA test revealed no significant difference in the average amount of contamination residues on the occlusal surface among the five groups ($p= 0.26$) However, a significant difference was observed in the average level of contamination residues on the body surfaces among the five groups ($p < 0.001$) (Table1). Furthermore, the Tukey post-hoc test demonstrated that the average level of contamination on the body surfaces was significantly lower in the air polisher and sodium hypochlorite groups compared to the other three groups ($p < 0.05$). No significant difference was found between the air polisher and sodium hypochlorite groups, and there were no significant differences among the control, hydrogen peroxide, and chlorhexidine groups (Table2).

4. Discussion

Reusing HAs in daily clinical practice is common, primarily due to cost-effectiveness for both patients and clinicians. According to evaluations, the cost of an HA for manufacturing companies is approximately 15% of the price of an implant (Bidra et al., 2020). However, there are limitations, such as the risk of cross-contamination in patients, which restricts their application (Browne et al., 2012).The findings of this study revealed that both mechanical methods (air polishing) and chemical methods (using hydrogen peroxide, chlorhexidine, and sodium hypochlorite) were unable to completely eliminate residual contamination on HAs which is align with recent studies(Almehmadi, 2021; Chew et al., 2018).One concern in the re-usage of HAs, is prion

Table 1
The Average percentage of contamination residing on the occlusal and body surface in five groups.

Occlusal surface				Body surface		
Groups	Mean	SD	p-value	Mean	SD	p-value
Control	5.5	2.8	0.26	6.1	2.3	<0.001
Hydrogen peroxide	4.8	3.1		4.6	0.7	
Air polisher	3.1	1.8		1.7	1.1	
Chlorhexidine	5.3	2.5		5.4	2.4	
Sodium hypochlorite	4.9	2.5		2.4	1.1	

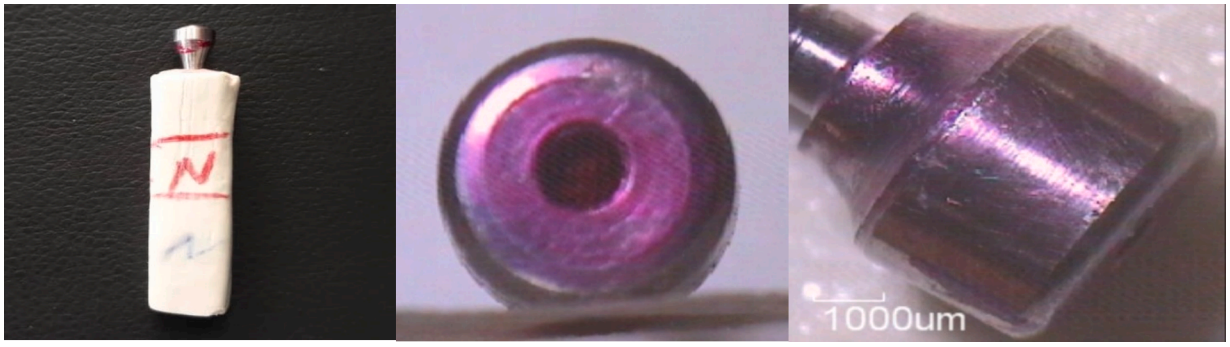

Fig. 3. Rectangular putty mold with four equal surfaces, images of the occlusal and body surfaces of HA.

Table 2
Pairwise comparison of the average percentage of residual contamination on body surfaces between groups using Tukey's Post Hoc test.

Groups	P-value
Control and hydrogen peroxide	0.28
Control and air polisher	<0.001
Control and chlorhexidine	0.88
Control and sodium hypochlorite	<0.001
Hydrogen peroxide and air polisher	0.003
Hydrogen peroxide and chlorhexidine	0.83
Hydrogen peroxide and sodium hypochlorite	0.04
Air polisher and chlorhexidine	<0.001
Air polisher and sodium hypochlorite	0.86
Chlorhexidine and sodium hypochlorite	0.002

contamination, which the risk of transmission in HA re-usage after the decontamination methods are very low, and it is more related to the re-reuse of endodontic files in the pulp in close contact with peripheral nerves(Eswaramurthy et al., 2022; Rapisarda, Bonaccorso, Tripi, & Condorelli, 1999). However, it is worth considering, the potential presence of prions on implant drills, which are typically considered reusable by implant manufacturing companies, as well as biomaterials sourced from animals such as cows(Bidra et al., 2020; Chew et al., 2018; Rapisarda et al., 1999).Furthermore, some studies have not regarded the reuse of HAs as an ideal procedure (Abreu et al., 2023; Sahin & Dere, 2021). However, they have suggested that by effectively applying mechanical or chemical methods to remove debris and contamination prior to autoclaving, promising results can be achieved (Almehmadi, 2021; Sánchez-Garcés, Jorba, Ciurana, Vinas, & Vinuesa, 2019). In a study conducted by Browne et al. (Browne et al., 2012), the used elements, including implant impression copings and healing abutments, exhibited sterility levels equal to new elements without any visible distortion after multiple rounds of sterilization using steam autoclave and Chemiclave protocols. Additionally, it was reported the levels of pro-inflammatory cytokines such as IL-1β and TNF-α in *peri*-implant crevicular fluid and clinical parameters like bleeding index and plaque index didn't show significant differences in patients using both unused and reused HAs (Lashkarizadeh, Foroudisefat, Abyari, Mohammadi, & Lashkarizadeh, 2022).

Based on the data obtained from this study, the null hypothesis that the amount of residual contamination in all groups and in all surfaces was the same, was rejected. Simultaneously, the findings of this study indicate no significant distinction among the five groups at the occlusal level. This observation could be attributed to the geometry and shape of the HA, influencing the accumulation of contaminants in different areas. It is possible that the limited access to the deep recesses within the occlusal part of the HA contributed to this outcome. This finding aligns with the results of a study conducted by Michelle Chew et al. (Chew et al., 2018), which demonstrated effective decontamination primarily on the body surfaces, followed by the bottom and then the occlusal surface. Furthermore, the results obtained indicate a significant difference in contamination levels on the body surfaces, with lower levels observed in the air polishing and sodium hypochlorite groups compared to the other groups. The residual contamination in the chlorhexidine and hydrogen peroxide groups was comparable to that of the control group. In a study evaluated the soft tissue response to clinically retrieved and decontaminated cover screws in a rat model, researchers reported that hydrogen peroxide, in conjunction with CO2 laser, could be clinically utilized for adequate decontamination of titanium surfaces, but not when used alone(Mouhyi, Sennerby, & Van Reck, 2000).

The positive impact of air polishing on removing residual organic contamination from HAs without causing harm was observed in this study, which is consistent with the findings of Chew's study (Chew et al., 2018). They found that using air polishing with erythritol powder effectively eliminated contamination. However, it is important to note that none of the decontamination methods employed should alter surface characteristics such as roughness, wettability, or surface energy. Nevertheless, the efficacy of air polishing as a decontamination method remains uncertain due to its potential impact on the surface characteristics and topography of healing abutments and needs further investigation (Louropoulou, Slot, & Van der Weijden, 2014).

The beneficial effect of sodium hypochlorite is likely attributed to the release of potent oxidizing agents, including free radicals. In addition to its disinfecting properties, sodium hypochlorite can dissolve organic residues on the HA surface (Abuhaimed & Abou Neel, 2017). Recent studies also reported the combination of sodium hypochlorite with electrochemical decontamination is more effective than sodium hypochlorite alone, and can remove soft and hard deposits, without altering the HAs surface topography and HA reuse can be considered multiple times in this combined decontamination protocol (Kyaw et al., 2023; Kyaw et al., 2020).It should also be acknowledged that variations in the effectiveness of sodium hypochlorite observed in different studies may be attributed to differences in concentration, duration of application, and application method.

When evaluating the reutilization of HAs, factors such as the type of healing abutment (titanium, stainless steel, zirconia, or polymer), the number of times the HA has been used, whether it was used on one patient or multiple patients, the duration of HA usage, and whether it was used in one-stage or two-stage surgery, as well as its use in guided bone regeneration procedures, should also be taken into consideration.

A limitation of this study was the small sample size, and the evaluation of residual contamination in the bottom surface of the HA was not conducted using the method employed. Additionally, the identification of non-protein residual contamination and the source of the remaining proteins was not evaluated. HA contamination differs from patient to patient based on diet, oral microflora, and oral hygiene, which come from saliva, food debris, and organic material like blood and epithelial cells (Rompen, Domken, Degidi, Farias Pontes, & Piattelli, 2006), making it difficult to standardize the type of prior use in different patients. Ultimately, the matter of sterilization and reuse of healing abutments continues to pose challenges in terms of safety, ethics, and cost. Consequently, further research and studies involving larger sample sizes are deemed essential to address this issue. Additionally, in future in vivo research, it is important to focus on the clinical implications related to the healing of soft and hard tissues and the assessment of biological complications.

5. Conclusion

In none of the studied groups, the contamination was completely eliminated in reused HAs. Additionally, the findings of this study indicate that incorporating sodium hypochlorite and air polishing, alongside autoclaving, can serve as an effective approach to minimize residual contamination on the body surfaces of utilized titanium HAs. Also, no significant difference was observed in the average amount of contamination residues on the occlusal surface among the five groups.

Author contribution

N.N developed the theoretical framework of this manuscript, performed and supervised the project, and contributed to the final version of the manuscript. A.H prepared the manuscript and summed up the data. A.M developed the theoretical framework of this manuscript. J.Y helped in writing the article. Z.P and N.KH measured the requested parameters and performed the project. All authors read and approved the final version before submission.

Ethics statement.

The present study was approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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