# In-Vitro Study of the Contamination Remaining on Used Healing Abutments after Cleaning and Sterilizing in Dental Practice

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## ABSTRACT

*Background:* Reuse or "recycle" of dental implant healing abutments in clinical practice is common, primarily for economic rational.

*Purpose:* To determine of this practice results in reuse of components that carry with them some degree of contamination between patients, even following thorough cleaning and sterilization.

*Materials and Methods:* One hundred healing abutments were collected from eight clinicians following patient use. The abutments were cleaned, sterilized, and then collected. The samples were treated with a protein specific stain (Phloxine B), and photographed.

*Results:* Ninety-nine percent of the abutments showed protein contamination at one or more sites following cleaning and sterilization.

*Conclusion:* Reuse of healing abutments between patients should be reevaluated in light of this data.

KEY WORDS: abutments, contamination, healing, reuse components, surface properties, titanium

## INTRODUCTION

The implant healing abutment (HA) is designed to serve several purposes: When composed of a biocompatible material such as titanium or titanium alloys it can support and allow the spread of the nonbony superficial soft tissues during healing, then maturation. It also protects the internal aspect (usually a screw thread) within the implant body from the impaction of debris during the osseointegration healing phase.

Once the implant has achieved adequate soft tissue maturation and osseointegration is clinically confirmed the HA is removed and ultimately replaced with a definitive abutment and prosthesis. HAs are generally designated by the manufacturer for single use, though it is common practice that many clinicians clean and sterilize this component, often re-using (recycling) it for economic reasons. Some companies also collect these used components, clean sterilize and repackage them for sale.[1] Studies have confirmed that titanium HAs can be adequately sterilized,[2] and in some instances the form of sterilization can produce an increase in soft tissue cell adhesion and spread over a clean titanium surface.[3,4] However, recent studies have indicated that some of these components may not be as clean or sterile as previously thought and questioned the safety of re-use.[5,6] HA component contamination comes from a variety of sources including: saliva, epithelial cells, food debris, blood. Although specific protocols have been developed, it proves rather difficult to effectively clean contaminated titanium surfaces, largely because of the

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**TABLE 1 HA from the "as received" Test Group: Numbers and Types**

| Implant Healing Abutment Manufacture/Type | Number of Specimens |
|---|---|
| Ankylos | 1 |
| Astra | 9 |
| Biomet 3i | 4 |
| Dentsply | 2 |
| Hiossen | 14 |
| Nobelbiocare | |
| Replace Replace trilobe | 39 |
| Conical connection | 10 |
| Internal cover screw | 2 |
| Branemark | 1 |
| Straumann | |
| Tissue level | 13 |
| Bone level | 5 |
| Total | 100 |

strong binding of proteins and amino acids.[7,8] If a clean, viable surface is not achieved with a used HA, then with secondary use in a healing site the epithelium and connective tissue attachment may be affected.

If the re-used HA has residual material on surfaces other than those in direct contact with the healing soft tissues,[7–10] further biological and mechanical consequences may result. These relate to the site at which contamination residue occurs. For example, the implant abutment junction (IAJ) may become contaminated with debris preventing the components fitting as intended. In implant systems that depend on intimate component fit to provide an IAJ seal this could promote greater bacterial colonization.[11,12] A clean screw thread is critical to the mechanical function of the implant abutment joint. Alterations could affect the friction of the screw as it is torqued to develop the proposed preload for the final abutment placement.[13] Furthermore, disease transmission may also result in cases where protein or amino acids are resistant to normal sterilization practices.[14,15] The purpose of this study was to evaluate used, cleaned and sterilized titanium HAs from eight different dental offices with respect to residual contamination, specifically proteins, and to identify if any particular type of HA or site was more susceptible to contamination remnants.

## MATERIALS AND METHODS

One hundred used HAs were collected from eight dental offices, Table 1. All the offices confirmed the re-use of HAs on occasion. The offices were informed on the purpose of the study which was performed according to the World Medical Association Declaration of Helsinki. None of the offices, clinicians, or patients involved were declared and were kept anonymous with regard to the HAs they provided for this study. The HAs had been reportedly cleaned by each office following retrieval according to the individual office protocols which were not disclosed with respect to brands of materials used. However, these included: Mechanical wiping with disinfection cloths, ultrasonic bath for between 10 and 60 minutes in various solutions, some used water, some alcohol. All offices confirmed the HAs were steam autoclaved. Specimens from different manufacturers and types of the abutments were collected and included: Ankylos (York, PA, USA), Astra (York, PA, USA), Biomet 3i (Warsaw, IN, USA), Hiossen (Seoul, Korea), Nobel Biocare (Zurich, Switzerland), and Straumann (Basel, Switzerland). After collection, the HAs designated as the test group were the "as received" group. These were initially inspected with unaided vision to determine if any debris or other damage or contamination could be detected. The abutments were recorded for manufacturer and type (where multiple styles existed), then photographed. For staining, each HA was placed in an individual plastic bag with 2 mL of Phloxine B stain[15] and sealed. The bags were then placed in an ultrasonic bath for 10 minutes. Once removed from the bath each abutment was rinsed in de-ionized water and allowed to air dry. The abutments were visualized with oblique light and photographs were made at the following sites; the main body (B), connection (C) to implant fixture, screw (S) thread shank, and the screw driver (D) engagement site. The body of the HA was rotated through 90° angles allowing four photographs to be made. The occlusal aspect of the abutment including the screw driver attachment site was also photographed. Finally, the implant attachment site including the screw threads were also recorded. A control group consisting of three brand new HAs (Nobel Biocare: Trilobe), were initially cleaned with an alcohol wipe, placed in an ultrasonic bath containing de-ionized water for 20 minutes,

**Figure 1** As received specimen, reportedly: Used, wiped, cleaned, and autoclaved.

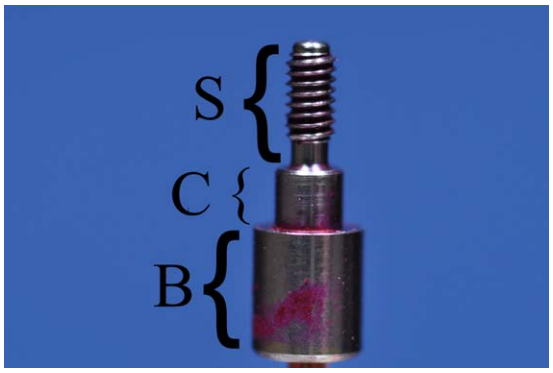


**Figure 3** Site evaluation: B- Body of the abutment – represents three sections, (Implant, mid, occlusal) all show phloxine B staining and residual contamination. C- Connection to implant site. S- Screw thread shank and threads.

then removed and steam autoclaved followed by the same staining protocols as the test group to evaluate the cleaning process and its effect with regard to the Phloxine B staining.

All photographs were examined by one examiner (CW) on a computer screen magnified at ×15. Recordings of the implant brand and the number of protein stained sites was recorded for each abutment aspect for both the "as received" group and the control group. The degree of contamination on the body (B) of the HA was quantified as it related to the vertical height of the abutment categorized in thirds (Implant site, middle, occlusal). The extent of contamination was also related to the implant brand to determine if any characteristics of the different brands played a role in the contamination site.

## RESULTS

Representative photographs of the specimens are seen in Figures 1–4. Although it was possible to see contamination with the unaided vison on some of the abutments, many (68/100) appeared to be free of residual contamination. Some of the abutments were color anodized on some or all surfaces which made evaluation prior to staining more difficult, however, after staining the site and quantity of the contamination was evident (Figure 5). The visualized results revealed a reddish-orange color in the areas of residual protein and amino acid contamination. All HAs except one showed at least one surface stained by the phloxine B and therefore contaminated (Table 2).

Of the 99 HAs showing contamination on at least one site: 92 had contamination on the (S) screw thread or shank of the screw. The implant connection (C) site had contamination on 85/99 of the healing abutments. Of the 400 body rotation sites recorded 46 were considered to be clean, with the remainder 354 surfaces showing some form of staining. The location of the contamination and numbers involved are listed in Table 2. Some variation in the site of residual contaminant staining was noted, but could not be further quantified due to the imbalance in sample size of abutment type. However, some

**Figure 2** Phloxine B protein and peptide stained. Sites of contamination seen red/orange.

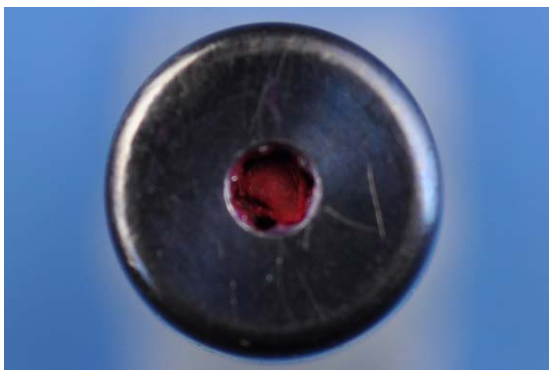

**Figure 4** Screw driver (D) engagement site with Phloxine B stain evident.

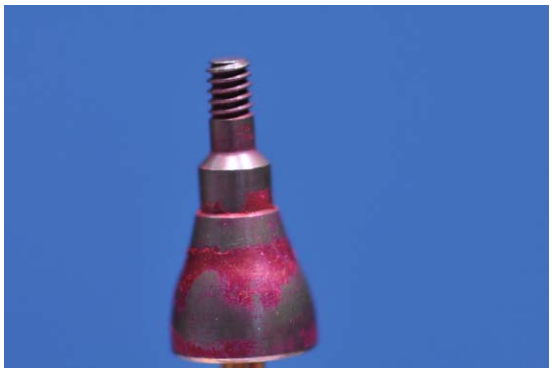

**Figure 5** Anodized healing abutment, the phloxine B clearly highlights residual contamination site.

generalizations could be made: It was noted that some implant healing abutments contained a groove circumferentially near the occlusal aspect of the body of the abutment which was consistently stained and would be expected to harbor debris as it is very improbable that this can be effectively cleaned (Figure 6). [Correction added on 16 February 2016, after first online publication: the name of the abutment "Hiossen implants" was used in the sentence in error. The last sentence has been changed from "It was noted that the Hiossen implants contained ... cleaned (Figure 6)" to "It was noted that some healing abutments contained ... cleaned (Figure 6)".]

The tapered form of implant abutment (Figures 2 and 5) had a tendency to show contamination at the IAJ, due to the inherent ledge design. Along the body of the abutment, when segmented into thirds (BO, BM, BI) there appeared to be little difference in the percentage of residual contamination sites (61–65%). The screw driver site (D) was contaminated in 90% of specimens (Figure 4). In contrast, none of the control group had any staining when evaluated at any of the sites (Figure 7).

## DISCUSSION

The practice of re-using implant components has been evaluated previously with respect primarily to the ability to provide a sterile component that provide an economical advantage to the patient or the clinician. Although many clinicians suggest this practice is performed for the patients' benefit it is not known how often a reduced fee is given. It is also not known how many implant surgeons recycle used healing abutments from one patient to the next, but unless these materials can be adequately cleaned and sterilized this practice should be reevaluated in light of the findings from this study for the following reasons: First, soft tissue integration is influenced by a material's characteristics.[7] In vitro, animal and human studies have all demonstrated titanium and titanium alloy with their biocompatible oxide layer to have the appropriate chemical composition allowing both epithelial cells and connective tissue fibroblasts to adhere, spread, and proliferate. Second, surface-free energy is seen to be high with a clean surface and conversely, low where a contaminated surface exists. The higher the surface free energy the better the wettability of the surface with respect to cell attachment and spreading. Surface texture can also have a profound effect. It has been demonstrated that epithelium and human gingival fibroblasts attach and spread more readily on polished surfaces, and that cells are sensitive to features as small as 0.2 $\mu$m.[8]

Contamination of a healing abutment is derived from several sources such as bacterial plaque, epithelium attached to titanium that tears during abutment removal, blood, food debris, and saliva.[7] These all contain proteins and amino acids that once adherent to titanium are extremely difficult to remove.[8]

**TABLE 2 Number and Site of Implant Contamination of "as received" Group**

| Healing Abutment Site Contaminated | Number of Contaminated Abutment Surfaces | Percentage of Contaminated Abutment Surfaces (%) |
|---|---|---|
| All implant healing abutments | 99/100 | 99 |
| Screw thread/shank (S) | 92/99 | 93 |
| Implant connection site (C) | 85/99 | 86 |
| Screw driver engaging site (D) | 89/99 | 90 |
| Body location; occlusal (BO) | 241/400 | 61 |
| Middle (BM) | 251/400 | 63 |
| Implant (BI) | 262/400 | 65 |

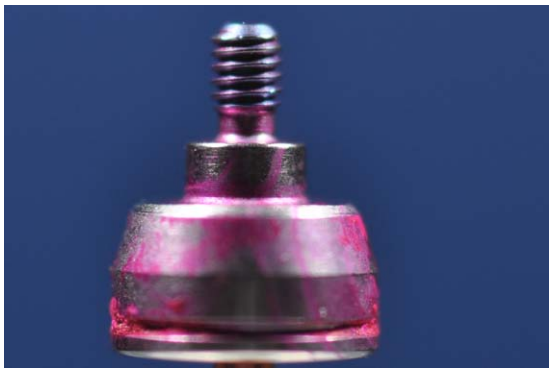

**Figure 6** Implant healing abutments that contained circumferential grooves showed contamination within the site.

**Figure 7** New healing abutment. Wiped, ultrasonic bath cleaned, sterilized, and stained with Phloxine B. No protein or peptide stained.

If these materials are not adequately removed prior to autoclave sterilization it is likely they will become baked onto the titanium surfaces. Phloxine B (Acid red 92, D &C 28) is a fluoresin derivative stain used as a protein and peptide highlighting stain. It is used extensively in forensics as a blood staining test and has uses for staining bacteria as well as being cleared by the FDA as a drug and food colorant.

One concern specific to finding proteins and peptide remnants on the surface of the used, clean and sterile healing abutments is the potential transmission of some biological elements that are not destroyed during normal sterilization processes preformed in the dental office. The prion protein core is highly resistant to proteolytic enzymes, is a small molecule that is filterable, can survive dry heat at 200°C for 1 to 2 hours, and when fixed by desiccation or chemicals may retain infectivity for years.[14,15] The clinical significance of the transmission of pathogenic prions that remain viable following commonly practiced dental sterilization also needs to be carefully weighed against the minor economic benefits of the re-use of healing abutments between patients.

The site of contamination may also affect the mechanical properties of the implant abutment connection. A contaminated screw thread preforms very differently to a clean one with regard to friction as it is tightened. When torque is applied to the screw, an increase in friction reduces the level of preload developed within the screw threads, which in turn reduces clamping forces related to the implant abutment joint.[13] Contamination within the implant body itself is also a concern, as it is known to harbor microbes that may contribute to peri-implant disease.[11,12] Some implant abutment joints have interfaces designed to provide a hermetic seal to prevent microbial colonization. The seal is dependent on the direct contact of the abutment to the implant, contaminants may result in a failure to produce such a seal.[12]

Limitations of this study include the identification of the residual contaminants other than being comprised of proteins or peptides, or even where the proteins were derived from. They could have been bacterial contaminants, host cell adherent material, or even food debris. This would require further study with more advanced genomic testing.

Also insufficient information on how many times each abutment may have been re-used would have been useful. For example, the one test abutment that showed no protein residue may well have never been used on a patient. This was speculated on in the light of that all other implants had residue identified on them. Other brands of abutments would also have been useful, especially those that had modified or roughened surfaces to determine if they would have more residual protein contaminants. Another limitation was that only titanium alloy abutments were evaluated, other materials are used for healing abutments. However, it is not known if these are recycled or even how they are cleaned and sterilized.

## CONCLUSIONS

The cleaning and sterilization of healing abutments performed in dental practice does not result in complete removal of contaminants. Proteins and peptides remain on 99% of specimens tested. The economic value of healing abutment re-use or recycle should be considered against all the potential risks and detrimental effects.

## DISCLOSURE

The authors declare no potential conflict of interest with respect to the authorship and/or publication of this manuscript.

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