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ORIGINAL ARTICLE

Prevention of internal bacterial colonization of dental implants: A comparative longitudinal observational study

Pia-Merete Jervøe-Storm[1] | Søren Jepsen[1] | Michael Marder[2] | Dominik Kraus[2] | Milan Stoilov[2] | Norbert Enkling[2,3]

[1]Department of Periodontology, Operative and Preventive Dentistry, University Hospital Bonn, Bonn, Germany

[2]Department of Prosthodontics, Preclinical Education and Dental Material Science, University Hospital Bonn, Bonn, Germany

[3]Department of Reconstructive Dentistry & Gerodontology, University of Bern, Bern, Switzerland

Correspondence
Pia-Merete Jervøe-Storm, Department of Periodontology, Operative and Preventive Dentistry, Rheinische Friedrich-Wilhelms-University Bonn, Welschnonnenstraße 17, 53111 Bonn, Germany.
Email: storm@uni-bonn.de

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Abstract

Objectives: Previous studies have indicated a progressive internal bacterial colonization of implants and possible implications for peri-implant bone loss. The aim of this study was to evaluate a decontamination protocol, two disinfectants, and a sealant for their ability to prevent such a colonization.

Materials and Methods: Bacterial samples were harvested from the peri-implant sulcus (external) and following abutment removal from the implant cavity (internal) during routine supportive peri-implant care in 30 edentulous patients 2 years after they had obtained two implants. In a split-mouth design, implants were randomly assigned to receive either internal decontamination alone (10% H_2O_2, brush) or additional placement of either sealant (GS), disinfectant agent (CHX-varnish) or disinfectant gel (1% CHX-gel), in the internal cavity before remounting of abutment/suprastructure. Twelve months later, internal and external sampling was repeated. Total bacterial counts (TBCs) were determined using real-time PCR in a total of 240 samples (eight per patient).

Results: Total bacterial counts in the internal cavity significantly reduced overall treatment modalities 1 year after the treatments (4.0 [2.3–6.9]-fold reduction; $p = .000$). No significant differences between the four treatment types were found ($p = .348$). Comparison of internal and external sampling points revealed significant correlation ($R^2 = .366$; $p = .000$) with systematically higher TBC counts in external samples.

Conclusions: Within the limitations of the present study, it can be concluded that the use of disinfectant agents or a sealant did not show an additional benefit in the prevention of internal bacterial colonization of implants compared to a decontamination protocol alone.

KEYWORDS
biofilm, decontamination, dental implant, hydrogen peroxide, peri-implantitis, sealing

1 | INTRODUCTION

The replacement of teeth with dental implants is nowadays recognized as standard treatment, helping patients with tooth loss to have function, esthetics, and phonetics restored (Buser et al., 2017). Not only advantages but also complications linked to the treatment with dental implants have been described (Buser et al., 2017). Possible failures are classified into two groups, seen as biological or technical complications (Berglundh et al., 2002). The main etiological cause of biological complications, mostly peri-implant diseases, is the colonization of the dental implant with microorganisms (Daubert & Weinstein, 2019).

This biofilm formation on the implant surface can result in microbial dysbiosis adjacent to the soft tissues (Berglundh et al., 2019; Heitz-Mayfield & Salvi, 2018; Kröger et al., 2018; Schwarz et al., 2018).

Numerous studies suggest that microorganisms are the major important etiological factor not only for the development but also for the progression of peri-implant inflammation (Heitz-Mayfield et al., 2020). This refers to a pathological condition in the tissues around a dental implant, which is subdivided into peri-implant mucositis and peri-implantitis (Berglundh et al., 2018, 2019; Heitz-Mayfield & Salvi, 2018; Jepsen et al., 2015; Schwarz et al., 2018).

Internal bacterial colonization within a two-piece implant resulting from a microbial microleakage in the microgap between implant and abutment/suprastructure of screw-retained fixed restorations has been described (Cosyn et al., 2011; Scarano et al., 2005). The microbial colonization of the internal cavity of the implant results in a bacterial reservoir (Penarrocha-Oltra et al., 2016; Romanos et al., 2016; Scarano et al., 2005). This microgap does allow microorganisms and their metabolic products to pass from the oral cavity to the internal cavity of the implant and vice versa (Cosyn et al., 2011; Steinebrunner et al., 2005). Previous studies of our group have shown an increase in bacteria in the microgap at the implant–abutment junction over a period of 12 months that was associated with a peri-implant bone loss extending the accepted rate of bone remodeling within the 2 first years after insertion (Enkling et al., 2011; Jervøe-Storm et al., 2015). A systematic review reported significantly higher bacterial counts for periodontal pathogenic bacteria within the implant–abutment interface of implants in patients with peri-implantitis compared to those implants surrounded by healthy peri-implant tissues (Tallarico et al., 2017).

Recently, we evaluated protocols for the decontamination of the internal cavity of two-piece implants. A comparison of four different irrigation solutions demonstrated that 10% hydrogen peroxide (H_2O_2) performed best for internal decontamination, both in vitro and in vivo (Jervøe-Storm et al., 2021).

The aim of the present study was to evaluate the long-term effect (over 12 months) of this decontamination protocol, alone or in combination with disinfectants or a sealant for the prevention of internal bacterial colonization as part of supportive peri-implant care.

2 | MATERIALS AND METHODS

2.1 | Study design

The present longitudinal observational study was part of a prospective longitudinal evaluation of implants (Abou-Ayash et al., 2020; Enkling et al., 2021). Thirty edentulous healthy patients, each with two SICace® implants (SIC invent; length 9.5 mm, diameter 4 mm) that had been placed in the interforaminal region of the mandible, volunteered for the study. This two-piece implant has an internal hex connection with an interlocking clearance fit (Zipprich et al., 2007) and a medium-rough, sand-blasted, acid-etched surface including the implant collar. The implant shoulder had been placed epicrestally. All 60 implants were 2 years in function, mounted with two-implant SFI bar-retained mandibular overdentures (Abou-Ayash et al., 2020; Enkling et al., 2021). All patients participated in a regular supportive peri-implant care (SPIC) program.

The study was conducted along the European directives and ICH Harmonized Tripartite Guideline E6: Note for Guidance on Good Clinical Practice, CPMP/ICH/135/95 Step 5 (http://www.ema.europa.eu/ema/) as well as to the guidelines of Helsinki (2013 Brazil). All patients signed an informed consent prior to inclusion in the study. The study protocol had been reviewed and approved by the Cantonal Ethics Committee of Berne, Switzerland (KEK No 157/08).

2.2 | Subject population

Thirty edentulous patients (16 women/14 men; mean age 65 years [SD 11.8]) contributed with two implants each. Apart from their edentulism of at least 6 months or more at the time of inclusion in the study 2 years earlier, all patients had to be in good general condition. Medications influencing bone metabolism, dental anxiety, or drug abuse were reasons for exclusion. Further information about surgery, superconstructions, etc. can be found in the previous publications of the present study (Abou-Ayash et al., 2020). All patients came for routine maintenance of their implants and reconstructions. Peri-implant health was assessed by recording probing depths (PD in mm) and bleeding on probing (BOP; +/−) at four sites (Renvert et al., 2018). Plaque was measured at the same four sites (mesial, labial, distal, and oral) as PD and BOP (Löe, 1967). Before removal of the suprastructures and abutments, but after external sampling, all patients rinsed their mouth for 2 min with 0.2% chlorhexidine (Corsodyl, GlaxoSmithKline), followed by debridement and cleaning of the suprastructures. All clinical recordings were collected by a blinded examiner after external sampling.

2.3 | Microbiological sampling and internal decontamination

Before sampling, implants and their adjacent regions were isolated with cotton rolls; great care was taken to avoid any kind of

contamination of the implants with saliva or blood during removal of the internal screw. Similarly, this was also maintained during internal irrigation, brushing, and during sampling. Bacteria were sampled as follows: paper point samples (#90; Co. Roeko) were obtained from all implants as described previously (Enkling et al., 2011; Jervøe-Storm et al., 2015). Sampling was first performed externally in the sulcus on the exterior of the implant ($t1_{[ext]}$). At this point in the 2nd year of the study, when implants had been in function for 2 years, the SFI bar and implant adapters were removed. The tube of the SFI bar was replaced by a new tube because we aimed to examine the wear of the tube (male part) and the matrices after 2 years in use. The implant adapters and the ball joints, as well as the screws of the ball joints, were stored separately for >10 min (the time required for the study procedure) in 0.1% CHX solution and dried before being used again. After the suprastructures and abutments were removed, the first internal sampling took place at the same implants ($t1_{[int]}$). Subsequently, at all implants, decontamination was performed as follows: the internal cavity of the implant was irrigated with 10 mL 10% H_2O_2, followed by brushing with an interdental brush with a wire diameter of 0.6 mm and an effectiveness of 2.2 mm (Curaprox prime CPS 06, Curaden Germany GmbH). Then, another 10 mL of the 10% H_2O_2 irrigation solution was used (Jervøe-Storm et al., 2021).

Subsequently, the two implants of each patient were randomly assigned/attributed to either test or control group by use of a randomization table.

- Implants in the control group received no further treatment after basic decontamination.

Implants of the test groups received

- A sealant (GS; GapSeal; H&W; highly viscous silicone matrix with thymol),
- A disinfectant varnish (CHX-varnish; Cervitec Plus; Ivoclar Vivadent; chlorhexidine 1%/thymol 1%), or
- A disinfectant gel (CHX-gel; Corsodyl; GlaxoSmithKline; CHX-gel 1%),

placed in the internal cavity. Abutments and suprastructures were then mounted again. Twelve months after the treatment, internal and external bacterial sampling were repeated by a blinded clinician ($t2_{[ext]}$ & $t2_{[int]}$).

2.4 | Microbiological analysis

Each sample was inserted in a sterile transport tube and sent for later analysis to the same specialized blinded laboratory as in our previous studies (Jervøe-Storm et al., 2015, 2021). Total bacterial counts (TBCs) were determined using real-time PCR (Carpegen® Periodiagnostics, Carpegen) as described earlier (Jervøe-Storm et al., 2005). TBCs were determined with a universal probe; in this case, conserved r-DNA sequences. The primers for the determination of TBC in the real-time PCR-based test are designed in such a way that all eubacteria are specifically detected by binding the primers and the probe to the conserved regions of the ribosomal DNA. The conversion of qPCR results into concrete cell numbers is based on considerations that take into account the number of rDNA genes and genomes per cell. In addition, TBC was validated with defined cell numbers and has a coefficient of variation of 15% at maximum. The commercially available test used in the present article is validated, also for TBC. It is ensured that no fungal or human DNA will be detected. This analysis method has a level of detection of 10^4 for TBC (Jervøe-Storm et al., 2005).

2.5 | Statistical analysis

The statistical plan comprised the testing of following hypotheses:

- The various test compounds are superior to control (decontamination alone) in reducing TBC ($t1_{[int]}$ vs. $t2_{[int]}$).
- The various test compounds (GS, CHX-T, and CHX-GEL) will show different reductions in TBC ($t1_{[int]}$ vs. $t2_{[int]}$).
- Sampling for TBC on the peri-implant sulcus will reflect the results of sampling in the internal implant cavity (internal vs. external).

The primary outcome was the reduction in TBC. All tests were performed at a significance level of $\alpha = .05$. Because of the bounded nature of the data, the $\log_{10}$ values of TBC were used for statistical analysis. A repeated measures mixed model with *patient* as subject variable and *time* as within-subject variable was used to account for the split-mouth design. *Treatment type* and *time* (pre–post), as well as their interaction, were set as fixed factors. To account for the differences in the response of patients over time, *time* was also modeled as random within-subject factor to include a random slope. Interaction of *time* × *treatment type* was regarded as primary outcome in the model. Effect estimates were calculated by Satterthwaite approximation for unbalanced data. For multiple comparisons, sequential Bonferroni was used. Unless otherwise stated, values are noted as estimated mean [95% confidence intervals].

For evaluation of correlation between external and internal samples, linear regression was used with external measurements as the predictor variable.

The software IBM SPSS 27.0 (IBM) was used for the analysis. The present study was reported in compliance with the STROBE guidelines accessible through the EQUATOR network.

3 | RESULTS

All patients were available for examinations at both t1 and t2. No patient reported any discomfort or had any complaints about the study-related procedures. At t1, 2 years after implant placement, none of the implants were affected by peri-implantitis, as confirmed by radiographs. At the same time point, mean probing depth was 2.3 mm (SD 0.40) and mean PII was 0.5 (median: 0, range 0–1). BOP was

negative at all sites. One year later (t2), these parameters remained unchanged as to PD (2.3 mm; SD 0.59) and slightly increased for PII (mean 0.6, median: 1, range 0–1), with no signs of peri-implantitis. In terms of peri-implant mucositis, BOP was positive for six implants in five patients. One patient had both implants positive (GS and control), and the other four had only signs of peri-implant mucositis at the control implant. All patients had adhered to the SPIC program.

Both treatment and control procedures resulted in significantly reduced total bacterial counts measured in the implant internal cavity 1 year after the treatments ($logTBC_{t1}$: 6.70 [6.57–6.84] vs. $logTBC_{t2}$ 6.10 [5.97–6.23]; $F = 27.675$; df = 1, 30; $p = .000$; Figure 1), which roughly translates to a fourfold reduction in TBC. The type of treatment, however, exhibited no significant effect on the reduction in TBC between t1 and t2 ($F = 1.122$; df = 3, 58; $p = .348$) (Figure 1). Accordingly, no significant differences in TBC were detected between the four treatment types 1 year post-treatment at t2. Despite the majority of samples exhibiting reduced TBC after the treatments, there was a subset of implants spread over all treatment types that showed increased TBC at t2 when compared to t1 (Figure 2), almost resembling a dichotomous response. This type of response appeared to be patient specific, as most commonly, the increase in TBC was observed in both control and test implants within one patient (see red patients in Figure 2).

However, the causative factor leading to such response remains elusive as there was no visible dependency on any of the recorded parameters (*treatment type*, *age*, *gender*, *BOP*, *PD*, and *PII*). Altogether, 14 implants in eight patients showed elevated levels of TBC at T2. Of those, 11 implants (each 2 GS, CHX-varnish, and CHX-gel; 5 control) in six patients had no signs of peri-implant inflammation at all. The remaining three implants were distributed to two patients. Two were found in one patient, one implant (control) with peri-implant mucositis and one (GS group) without, and the last one (control group) in one patient presented with peri-implant mucositis. However, no change in probing depths at any implant was found from t1 to t2. Additionally, radiographs at t2 showed no signs of increased peri-implant bone loss, that is, peri-implantitis.

The comparison of measurements taken from the implant cavity (internal) and the peri-implant sulcus (external) revealed systematically higher bacterial counts in the external samples and the measurements were significantly correlated ($F = 10.05$; df = 1, 118; $b = 2.26$; $m = 0.605$; $R^2 = .366$; $p = .000$).

4 | DISCUSSION

This longitudinal observational study showed a significant reduction in the total bacterial counts in the internal implant cavities by more than 70% following the decontamination procedures. The additional application of a sealant or disinfectant did not lead to a greater reduction in TBC than decontamination with hydrogen peroxide alone. To the best of our knowledge, this study is the first study to investigate the effects of internal implant decontamination with or without a sealant or disinfectants in vivo over an observational period of 12 months. The results not only exceed the findings of our previous feasibility study that not only showed a mean TBC reduction of about 50% directly after the decontamination procedure (Jervøe-Storm et al., 2021) but also demonstrate a sustained effect over a period of 12 months.

The aim of the present in vivo study was to prevent the colonization of the inner cavity with the help of three different compounds. The results after 12 months of observation showed that a two-piece implant is still susceptible to bacterial colonization even after trying to seal the marginal gap between abutment and implant. Apparently, microleakage occurs due to loading forces when the implant is in function and cannot be completely prevented (Mishra et al., 2017; Steinebrunner et al., 2005).

Various attempts have been made to seal the microgap with different materials such as varnishes, composites, silicones, and

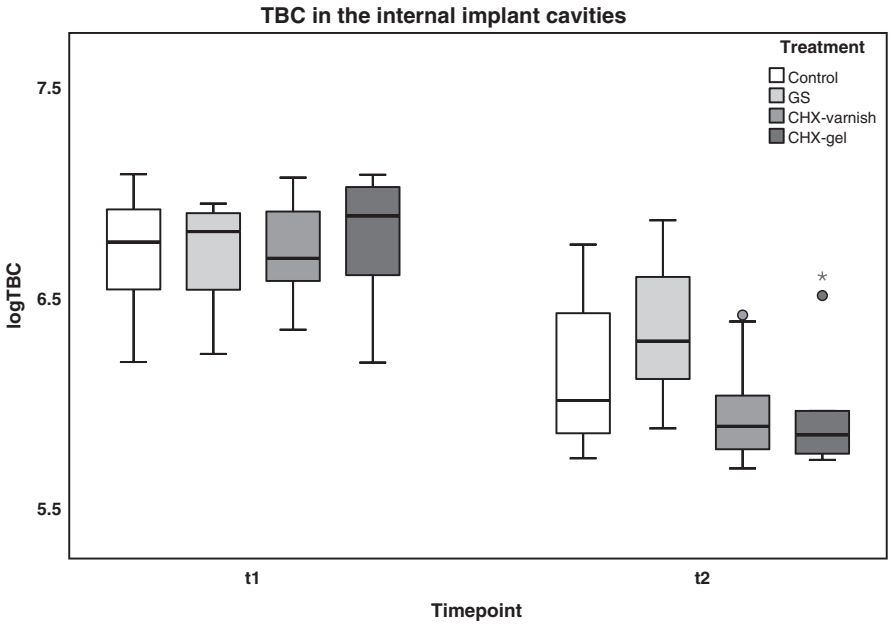

FIGURE 1 Boxplots showing the log TBC in samples from the internal implant cavity for the different treatments at t1 and t2. GS: sealant—highly viscous silicone matrix with thymol; CHX varnish: chlorhexidine 1%–thymol 1% varnish; CHX-gel: chlorhexidine gel 1%; and control: decontamination alone (decontamination with H_2O_2 and a brush). t1: Baseline (implants 2 years in function); t2: 12 months later.

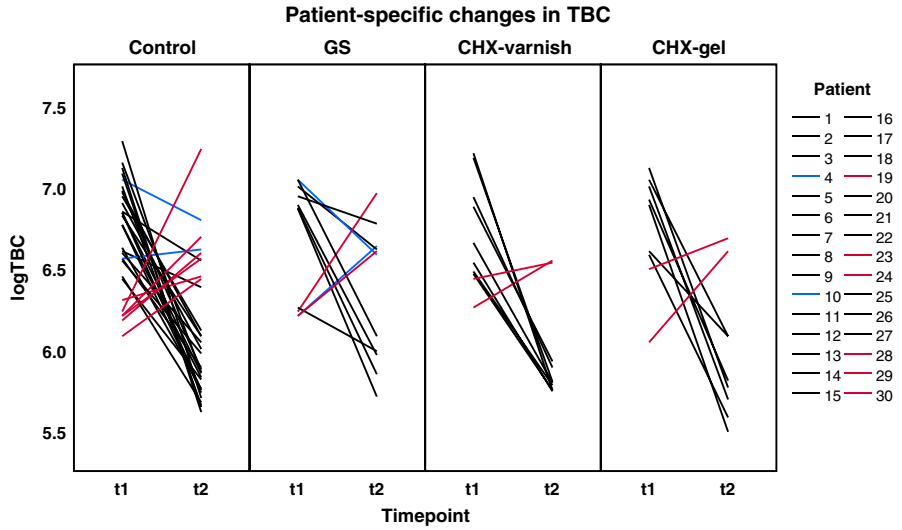

FIGURE 2 Development of logTBC measured in the internal implant cavity between t1 and t2 for the different treatments. Each trajectory shows the change in TBC for individual implants. Trajectories of patients where both implants (control and test treatment) exhibited higher TBC at t2 are highlighted in red; patients with both treatments resulting in lower TBC are colored in black; and patients with mixed reactions are identified by blue trajectories. GS: sealant—highly viscous silicone matrix with thymol; CHX varnish: chlorhexidine 1%–thymol 1% varnish; CHX-gel: chlorhexidine gel 1%; and control: decontamination alone (decontamination with H_2O_2 and a brush). t1: Baseline (implants 2 years in function); t2: 12 months later.

gutta-percha (AlQarawi et al., 2021; Carinci et al., 2019; Duarte et al., 2006; Paolantonio et al., 2008; Proff et al., 2006).

A systematic review analyzed the effects of various materials to prevent infiltration of the internal cavity (Alves de Sousa et al., 2020). Based on eight in vitro trials, they concluded that using a varnish/coating was efficient, especially when combined with a physical barrier such as PFTE. Of the included studies, only four examined sealing of the implant–abutment interface (Cardoso et al., 2016; Duarte et al., 2006; Nayak et al., 2014; Proff et al., 2006). Of those four trials, two examined the compounds as used in the present study. One study compared the same chlorhexidine–thymol-based disinfectant (CHX varnish) used in the present study with a silicone sealant. *Enterococcus faecalis* was used for contamination; neither of the two materials showed an effective sealing after 63 days (Duarte et al., 2006). The other trial compared the effect of a sealant (GS) with that of an O-ring using cultivation after 5 days of incubation with *enterococci* and found microbial growth in both groups (Nayak et al., 2014). There was a slightly lower growth in the GS group.

A recently published in vitro study on 100 dental implants compared the sealant used in the present study (GS) with a gel based on active oxygen technology with antimicrobial activity, a sealant based on a polydimethylsiloxane matrix with the addition of thymol, a negative control (no sealing), and a positive control group (chlorhexidine gel) (Smojver et al., 2022). The different groups were exposed to a mixture of strains of *Candida albicans* and *Staphylococcus aureus* for 14 days. Samples from the inner cavity of the implants were obtained and incubated for 48 h, and identification of the resulting colonies was performed with a MALDI biotyper. Only the sealing showed significantly better results than the negative control.

Because of their in vitro design and the short observation periods, the results of these three studies cannot be compared with the findings of our study.

Only a few in vivo studies have addressed the topic of the present investigation. Carinci et al. (2019) studied coating of the inner cavity of dental implants with an alcoholic solution containing polysiloxane oligomers and chlorhexidine gluconate 1% and showed that TBC obtained from treated implants was lower compared to TBC from untreated implants. However, this observation was made after 10 days of exposure of the implant to the oral cavity 4 months after insertion. In another in vivo study (Paolantonio et al., 2008), the efficacy of a 1% chlorhexidine gel on internal bacterial contamination of implants with screw-retained abutments was investigated over 6 months. It was shown, based on TBC analyzed with cultivation, that CHX gel in a semi-closed system, such as the inner implant cavity, effectively reduced the bacterial population. In the present study, the chlorhexidine-based disinfectants did not provide a significant benefit; decontamination alone was enough.

The present investigation has certain strengths but also limitations. The fact that we investigated a very homogeneous patient/implant population can be seen as a strength of our study. All patients were edentulous and had been treated with the same two-implant SFI bar-retained mandibular overdenture. The SFI bar consists of a massive abutment (implant adapter) that overcomes the mucosal height – the abutment is one piece and thus has no possibility of contamination of the implant interior by an occlusal screw. Due to the design, there was no occlusal access hole that would have reached into the interior of the implant. All implants were produced by the same manufacturer, inserted by the same surgeon, and had been in function for 2 years. Our study had a clear rationale that was based

on our earlier observations and extended these previous findings in a longitudinal prospective fashion. In contrast to other investigations, the long observation period of 12 months is a clear advantage and of practical value for its clinical implications as it mimics the clinical reality. In clinical practice, abutments and suprastructures are usually not removed and cleaned more frequently than once a year.

All implants had been in function for 2 years during which time an undisturbed internal bacterial colonization occurred as has been previously reported for various two-piece dental implants (Canullo et al., 2015; Enkling et al., 2011; Jervøe-Storm et al., 2015). Based on our previous analyses (Jervøe-Storm et al., 2015), using the same implant system, it can be assumed that the internal implant cavities had been sterile at implant insertion. An open implant surgery was performed with a full-thickness flap. As always, great care was taken to ensure that no blood or saliva got into the inside of the implant. There was no second-stage surgery, but the healing abutments were replaced by adapters after 3 months (Abou-Ayash et al., 2020; Enkling et al., 2021). Furthermore, the amounts of TBC from internal and external samples correlated well, which supports the repeatedly demonstrated and reported concept of communication (microleakage) between internal and external peri-implant regions. The findings of the present investigation could demonstrate for the first time that the dynamics of such progressive bacterial colonization could be altered by a one-time decontamination intervention with effects for up to 12 months.

Obviously, the present study has also some limitations. The homogeneous implant sample with all its aforementioned advantages may limit the generalizability of the obtained results because there is a wealth of different implant designs available. The effect of different implant designs against microleakage was investigated in a systematic review based on 30 studies (Mishra et al., 2017). Microleakage and internal contamination were found in variable amounts independent of implant–abutment design, and it increased under dynamic loading conditions.

The detection limit of the analytical method used in the present study, based on real-time PCR, is 10^4 bacteria/sample (Jervøe-Storm et al., 2005). This test does not distinguish between live and dead microorganisms. However, this may not be clinically important, as vital or nonvital microorganisms or even smaller molecules such as bacterial endotoxins in the implant can exert an influence on the immune system in the peri-implant environment through the implant–abutment connection (Harder et al., 2010, 2012; Koutouzis, 2019).

Furthermore, it is possible that the present study is underpowered to detect minor differences between the treatment types. The study population was limited to 60 implants in 30 patients from a longitudinal study, with control and experimental treatment administered for each patient to decrease the effect of large differences in the response between patients. Similar in vivo studies included 15 patients with 60 implants (Carinci et al., 2019) and 30 patients contributing with 1 implant each (Paolantonio et al., 2008). Five in vitro studies had 6 to 30 implants in their groups (Cardoso et al., 2016; Duarte et al., 2006; Nayak et al., 2014; Proff et al., 2006; Smojver et al., 2022). Thus, we feel comfortable about the amount of 30 included patients with two implants each in the present in vivo study. It must be stated that the effect size was difficult to estimate a priori, as there are no studies available comparing the here applied treatments over a longer period.

Interestingly, 14 implants in eight patients showed elevated amounts of TBC at t2. The frequencies of such findings were evenly distributed among the groups. One possible reason for this effect could be a minimal, clinically not detectable loosening of the abutment screw, allowing more bacteria to penetrate into the internal cavity. The observation that the effect might be patient specific, however, suggests the influence of a yet unrevealed predictor variable. This assumption is substantiated by the correlation of external and internal TBCs and by the fact that patients exhibiting higher internal TBC at t2 in most cases also showed higher external TBC. A contamination from the superstructures could not have taken place, as the SFI bar was replaced and adapters and screws were decontaminated with 0.1% CHX for >10 min before reuse.

With regard to the study design, an additional control group without any intervention could have been added. This would have helped in understanding which effects sealants and disinfectants add to H_2O_2 alone. However, we found this to be an ethical problem, as previous studies have indicated an association of bacterial internal colonization by pathogenic bacteria with peri-implant bone loss (Canullo et al., 2017; Jervøe-Storm et al., 2015). Taking into consideration that we have previously shown a connection between the use of 10% H_2O_2 and internal bacterial numbers, we, therefore, regard this treatment as a clinical standard in our trials (Jervøe-Storm et al., 2021).

Finally, no bacterial samples could be harvested directly after decontamination to serve as a new baseline for interpretation of the subsequent colonization dynamics. Therefore, at this point, one can only speculate whether the TBCs 12 months after decontamination are a result of a bacterial persistence or a recolonization or a combination of both. Future studies are required to address this question.

Nevertheless, compared with the findings at t1, the outcomes 12 months later generally indicate a favorable effect of the intervention.

In summary, a relatively simple and inexpensive decontamination procedure of the internal implant cavity with 10% hydrogen peroxide had a significant effect on the bacterial load that persisted for up to 12 months in the majority of implants studied. The additional use of a sealant or disinfectant had no significantly added benefit. The proposed decontamination protocol could become part of a routine supportive peri-implant care program.

AUTHOR CONTRIBUTIONS

PMJS and NE conceived the idea; NE collected the data; MM calculated the statistics; PMJS and SJ performed the data analysis and led the writing; DK, MS, and NE reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Pia-Merete Jervøe-Storm https://orcid.org/0000-0003-4697-6984
Søren Jepsen https://orcid.org/0000-0002-4160-5837
Michael Marder https://orcid.org/0000-0002-1365-7170
Norbert Enkling https://orcid.org/0000-0002-4698-8101

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