Prevention and treatment of peri-implant diseases

Cleaning of titanium dental implant surfaces

Louropoulou, A.
Chapter 6

The effect of chemotherapeutic agents on contaminated titanium surfaces: a systematic review

V.I. Ntouka
D.E. Slot
A. Louropoulou
G.A. van der Weijden

**Introduction**

Oral implantology is a dynamic field of modern dentistry. Dental implants have various indications and present high survival and success rates. Lambert et al. (2009) reported overall implant survival rates ranging from 94% (1 year) to 87.7% (15 years). Certain characteristics of the implant surface play a determining role in the longevity of the implants, with rough surfaces demonstrating higher success and survival rates than smooth surfaces (Lambert et al. 2009). It has been shown that surfaces with a roughness of approximately 1.5 mm, which corresponds to moderately rough implant surfaces, have the strongest biomechanical bond with alveolar bone (Albrektsson & Wennberg 2004).

On the other hand, rough surfaces may promote bacterial colonization and biofilm formation. Bacterial accumulation induces inflammatory changes in the soft tissues surrounding oral implants (peri-implant mucositis), which may lead to progressive destruction of the supporting bone (peri-implantitis), and ultimately, to implant failure (Esposito et al. 2006). Peri-implant mucositis, a reversible inflammation of the soft tissues surrounding a functional implant (Albrektsson & Isidor 1994), occurs in approximately 50% of all implants (Zitzmann & Berglundh 2008). Peri-implantitis is an inflammatory reaction associated with bone loss around a functional implant (Albrektsson & Isidor 1994) and affects from 12% (Fransson et al. 2005) to 43% (Roos-Jansäker et al. 2006) of peri-implant tissues. Astrand et al. (2004) reported a higher frequency of peri-implantitis for implants with a rougher surface. To avoid a bacterial shift towards more pathogenic flora, the use of a relatively smooth abutment and implant surface has been suggested (Quirynen et al. 2002).

There is insufficient evidence concerning the most effective intervention for the treatment of peri-implant diseases (Esposito et al. 2008) despite several attempts to determine the optimal treatment protocol for the complete resolution of peri-implantitis (Claffey et al. 2008). Renvert et al. (2009) reviewed the literature for evidence of any re-osseointegration of previously contaminated implant surfaces. The authors concluded that no method could predictably accomplish the complete resolution of the peri-implant defect. Although there is evidence that some treatments can be effective against peri-implantitis, the most effective intervention methods are presently unknown. Furthermore, among the interventions with similar degrees of effectiveness, the available research does not identify the treatments with fewer side effects, or those that are simpler and cheaper to use (Esposito et al. 2008).

The removal of bacterial deposits and the reduction of micro-organisms to a level compatible with health is the first step in the treatment of peri-implant diseases (Lindhe & Meyle
Because the available evidence for combination treatments is inconclusive (Claffey et al. 2008; Esposito et al. 2008), it is wise to examine the effectiveness of single treatments. Mechanical treatment alone is incapable of removing bacterial biofilms due to the screw-shaped design and surface roughness of dental implants. Furthermore, the suprastructure of the implant often hinders the access of mechanical instruments (Renvert et al. 2008). Thus, the use of different chemotherapeutic agents has been proposed for the treatment of infected implant surfaces (Renvert et al. 2008). A recent systematic review evaluated different treatments of peri-implantitis in vivo. No single method of implant surface decontamination was found to be superior (Claffey et al. 2008). Most of the studies included in recent reviews (Claffey et al. 2008; Esposito et al. 2008; Renvert et al. 2009) were not controlled or evaluated a combination rather than a single treatment. Furthermore, those studies did not assess the decontamination of implant surfaces but instead determined the effectiveness of each treatment based on cumulative parameters such as clinical outcomes. To identify the most effective chemical treatment, controlled studies with outcome variables related to the reduction of microorganisms on contaminated titanium surfaces are needed. Therefore, the aim of the present review was to systematically collect the available evidence, and based on the associated findings, evaluate the ability of different chemotherapeutic agents to decontaminate biofilm-contaminated titanium surfaces.

### Material and methods

**Focused question**

What is the efficacy of various chemotherapeutic agents in decontaminating biofilm-contaminated titanium surfaces as compared with a control?

**Search strategy**

Two internet sources were used to search for papers that met the inclusion criteria: the National Library of Medicine, Washington, DC (PubMed-MEDLINE) and the Cochrane Central Register of Controlled Trials (CENTRAL). Both databases were searched for studies conducted during or before June 2010. The search was designed to include any published study that evaluated the effects of chemotherapeutic agents on contaminated titanium surfaces. To achieve this goal, a wide and comprehensive search was performed. All possible treatment interventions for the decontamination of titanium surfaces were included, which ensured
the inclusion of papers that used treatment methods other than chemical solutions (but which may have provided chemical treatment as an alternative). All reference lists of the selected studies were handsearched for additional papers that might meet the eligibility criteria for inclusion in this study.

The following terms were used in the search strategy for both databases:

{Subject AND Adjective AND Intervention} 

\[\text{Subject: (Dental implant [MesH] OR Dental implant [textword])}\]

AND

\[\text{Adjective: (Smooth OR structure OR texture OR roughness OR surface OR biofilm OR plaque index OR dental plaque OR plaque OR dental depositn [textword])}\]

AND

\[\text{Intervention: (Ultrasonic OR curette OR scaling OR acid OR laser OR polishing OR debridement OR curettage OR chlorhexidine OR air abrasion OR cleaning OR cleansing agents OR instrumentation OR Ardoz-X OR decontamination OR citric acid OR phosphoric acid OR CPC OR Cetylpyridinium chloride OR SLS OR sodium lauryl sulfate OR EDTA OR ethylenediaminetetraacetic acid OR Chlorotetracycline OR Demeclocycline OR Doxycycline OR Lymecycline OR Methacycline OR Minocycline OR Oxytetracycline OR Rolitetracycline OR Tetracycline OR Tetracyclines OR Hydrogen peroxide OR H202 OR Sodium perborate OR Peroxyborate OR Peroxycarbonate [textword])}\]

The eligibility criteria:

- Controlled studies
- Standardized approach to the growth of biofilms on titanium surfaces
- Intervention: Treatment of contaminated titanium surfaces with a chemotherapeutic agent
- Evaluation parameters for surface decontamination: Residual biofilm, residual lipopolysaccharide (LPS), confocal laser scanning microscope (CLSM) or scanning electron microscope (SEM) observations
Screening and selection
Only papers written in English were accepted for further evaluation. Letters and narrative/historical reviews were not included in the search. Two reviewers (A.L & V.I.N) independently screened the papers for eligibility, first by title and abstract. If the search keywords were present in the title, the abstract was selected for reading. If the abstract was not present but the title contained keywords of interest or suggested that the article was related to the objectives of this review, the paper was also selected for full-text reading. In the case of disagreement, the opinion of a third reviewer (G.A.W) was decisive. Following selection, full-text papers were read in detail by two reviewers (G.A.W & V.I.N). Those papers that fulfilled all selection criteria were processed for data extraction. Disagreements were resolved by discussion. If disagreement persisted, the judgment of a third reviewer (D.E.S) was decisive. Two reviewers (G.A.W & V.I.N) hand-searched the reference lists of all included studies for additional papers.

Assessment of heterogeneity
Factors that were evaluated to assess heterogeneity across the selected studies were as follows:
- Titanium surfaces, contamination methods
- Chemical agents tested, concentrations, method and duration of application
- Outcome variables

Quality assessment
Two reviewers (D.E.S & V.I.N) scored the methodological quality of the studies selected for analysis. This assessment of methodological quality combined several proposed criteria (RCT-checklist of the Dutch Cochrane Center [2009], the MOOSE statement by Stroup et al. (2000), the STROBE statement by Von Elm et al. (2007), Esposito et al. (2001), Needleman et al. (2000), Verhagen et al. (1998), Jadad et al. (1996) and the CONSORT statement March (2010). Criteria were described for each of the three domains: external validity, internal validity and statistical methods. Each item was scored with either a “+” for an informative description of the issue and a study design that met the quality standards, “−” for an informative description but a study design that failed to meet the quality standards or “?” for lacking or insufficient information. A study was classified as having a low risk of bias when the surface material
was clinically representative; reproducibility data were provided; treatments were randomly allocated; preparation, manipulation and treatment of the surface were identical except for the intervention; point estimates were presented for the primary outcome measurements; and statistical analyses were described. Studies that lacked one of these six criteria were classified as having a moderate potential risk of bias and those that lacked two or more of these criteria indicated a high potential risk of bias (van der Weijden et al. 2009).

**Data extraction and analysis**

Data were extracted from the selected papers by two reviewers (D.E.S & V.I.N). Disagreements were resolved by discussion. If disagreement persisted, the judgment of a third reviewer (G.A.W) was decisive. After a preliminary evaluation of the selected papers, considerable heterogeneity was found in the study designs, characteristics, outcome variables and measurements. Furthermore, one out of the four studies had descriptive outcome variables. Consequently, it was not possible to perform a meta-analysis. Therefore, a descriptive summary of the data had to be adopted.

**Results**

**Search and selection**

The PubMed-MEDLINE and Cochrane-CENTRAL searches resulted in 2288 and 168 papers, respectively (Figure 1). In total, 2425 unique papers were found, and 31 papers were identical in both searches. The initial screening of titles and abstracts resulted in 12 full-text papers. After fulltext reading, eight papers were excluded. Table 1 shows the reasons for exclusion. Additional hand-searching of the reference lists of selected studies yielded no additional papers. Ultimately, four papers were processed for data extraction.

**Assessment of heterogeneity**

Information regarding the study characteristics is presented in Table 2. This table presents a short summary of the study design and the results of the selected papers. The considerable heterogeneity of these studies made comparisons between them difficult. Owing to the lack of uniform data presentation, the results of the studies could only be evaluated separately.
Titanium surfaces

Surface roughness is a determining factor in both biofilm formation and decontamination (Korber et al. 1997). The roughness of the titanium surfaces used varied among the studies. Zablotsky et al. (1992a) studied grit-blasted titanium surfaces that had an average surface roughness of 3.62 μm (Rønold et al. 2003). Machined and plasma-sprayed surfaces were used by Dennison et al. (1994). Titanium plasma-sprayed (TPS) surfaces display a roughness of 5.2 μm, according to Schwartz et al. (2001). Mouhyi et al. (2000) used commercially pure titanium foils with a textured surface of unknown roughness and finally, Chin et al. (2007) used machined surfaces with a mean surface roughness of approximately 182 nm.

The method of contamination also differed between the selected studies. Two studies used LPS from *Eschericia coli* or *Porphyromonas gingivalis* (Zablotsky et al. 1992a and Dennison et al. 1994, respectively). Mouhyi et al. (2000) used an *in situ* model to contaminate titanium foils by placing them in dentures in the mouths of volunteers. Finally, Chin et al. (2007) grew human saliva biofilms on titanium surfaces.

Treatment and outcome

Concerning the chemical agents tested, differences were observed in the concentrations and the methods and durations of application. Zablotsky et al. (1992a) and Dennison et al. (1994) used 0.12% chlorhexidine digluconate (CHX). Chin et al. (2007) also used CHX but at a higher percentage (0.2%). Citric acid was tested in a saturated (Dennison et al. 1994) or supersaturated (Mouhyi et al. 2000) solution. Zablotsky et al. (1992a) evaluated citric acid with a pH of 1, but the concentration was not mentioned.

Zablotsky et al. (1992a) and Dennison et al. (1994) burnished the chemotherapeutic agents on the titanium surface with a cotton pellet, whereas Chin et al. (2007) immersed the implant samples in the chemotherapeutic agents. Mouhyi et al. (2000) applied the chemicals with a pipette. The outcome variable for the first two studies (Zablotsky et al. 1992a and Dennison et al. 1994) was the residual radioactive LPS. Mouhyi et al. (2000) used SEM in their study, and Chin et al. (2007) used CLSM analysis to quantify the residual biofilm. In the CLSM analysis, the biofilm samples were sonicated and dispersed in demineralized water. Next, they were stained with a live/dead stain, and the remaining bacteria were enumerated (van der Mei et al. 2006).
Quality assessment

Quality assessments of the various studies reviewed are presented in Table 3. The estimated risk of bias is considered to be high for all four studies. The study by Mouhyi et al. (2000) did not fulfill any of the criteria established to determine quality, whereas the remaining three studies provided descriptions of the statistical analyses but did not report data concerning the reproducibility and did not randomly allocate the treatments. Representative titanium surfaces were used by Dennison et al. (1994) and Chin et al. (2007). Chin et al. (2007) did not carry out the preparation, manipulation and treatment of the surfaces identically except for the intervention because they used an untreated surface instead of a negative control. Dennison et al. (1994) did not present point estimates for the primary outcome measures.

Data extraction and analysis

Zablotsky et al. (1992a) used grit-blasted titanium alloy strips contaminated with *E. coli* LPS. In their study, 21 titanium strips were treated for 1 min with 0.12% CHX, 1.64% stannous fluoride, tetracycline HCl, 1% chloramine T, 3% saline, hydrogen peroxide (H₂O₂) or citric acid. The results are presented in Table 4. The residual LPS levels were measured by liquid scintillation spectrometry. Chloramine T, saline, H₂O₂ and citric acid treatments all resulted in lower LPS counts than the untreated controls. Stannous fluoride appeared to increase the LPS counts. Chloramine T and citric acid resulted in lower amounts of residual LPS compared with the saline control, but these differences failed to reach statistical significance.

Dennison et al. (1994) studied machined and TPS implants contaminated with radioactive *P. gingivalis* LPS. Three implants of each type were treated for 2 min with deionized water, saturated citric acid solution or 0.12% CHX. The results are presented as the percentage of the initial endotoxins removed (Table 5). The treatments (citric acid, CHX) were significantly more effective than the untreated control, but they demonstrated no statistically significant differences compared with deionized water (d-H₂O) in terms of their effectiveness on machined and plasma-sprayed surfaces.

Mouhyi et al. (2000) placed eight commercially pure titanium foils on dentures in volunteers. After 24 h in the volunteers’ mouths with no oral hygiene, the foils were collected and treated with supersaturated citric acid (three times for 30 s each), 10 mM H₂O₂ (2 min), or a combination of H₂O₂ (2 min) followed by citric acid (three times for 30 s each). Following all treatments, the discs were rinsed with ultrapure water. Eight non-contaminated, commercially pure titanium foils served as controls. SEM was used to assess the surface decontami-
nation. According to the authors, citric acid treatment resulted in a clean surface. However, some areas of bacterial contamination remained. \( \text{H}_2\text{O}_2 \) demonstrated no obvious cleaning effect. The combined treatment with citric acid and \( \text{H}_2\text{O}_2 \) resulted in some decontamination, but small dehydrated and burned debris remained attached to the surface. This study did not go beyond a descriptive analysis and provided no data.

Chin et al. (2007) used five commercially available, self-tapping micro-implants (pure titanium or titanium alloy) with machined surfaces. Human saliva was collected from 20 healthy volunteers. Saliva biofilms were grown on the implants for 20 h in an aerobic incubator. The contaminated implants were then treated with 0.2% CHX or 0.055% sodium fluoride mouth rinses for 1 min. Residual biofilms were sonicated and dispersed in demineralized water and stained with a live/dead stain, and the remaining bacteria were enumerated using a CLSM microscope. The data are presented in Table 6. Before the treatment, all of implants harboured an average of 57% viable microorganisms. The biofilms on the micro-implants treated with CHX and fluoride mouth rinses contained comparable numbers of viable organisms but significantly (80%) fewer viable organisms compared with the untreated micro-implants. Neither mouth rinse significantly reduced the number of bacteria. Thus, these mouth rinses kill but do not effectively remove bacteria from titanium implants.

In Table 7, we attempt to summarize the statistical analysis of the effects of various chemotherapeutic agents (versus their relevant controls) for the purposes of comparison. Among the different agents, the most data were available for citric acid and CHX. Three studies demonstrated a positive effect of citric acid on LPS and bacteria removal as compared with an untreated surface. However, one of these studies also compared citric acid with water treatment and did not establish a significant difference. The three studies that evaluated biofilm removal following the use of CHX showed no significant effect as compared with the control. However, Chin et al. (2007) noted the efficacy of CHX in bacterial killing.

**Discussion**

Although peri-implantitis is currently recognized as a distinct disease entity, the proposed treatments for this condition are still based on evidence obtained from the treatment of periodontitis. The rationale behind this practice is that the tissues surrounding dental implants are very similar to the tissues that surround the teeth (Berglundh et al. 1991). On the other hand, the titanium surface is dissimilar from the root surface and the direct application
of periodontal treatment measures to implants might be less effective. The screw-shaped
design and roughness of implant surfaces may facilitate biofilm formation during exposure
to the oral environment (Renvert et al. 2008) and may limit the effectiveness of mechanical
debridement (Karring et al. 2005). The available evidence suggests the use of a chemothera-
papeutic agent as an adjunct to mechanical therapy (Kozlovsky et al. 2006; Renvert et al. 2008).

Persson et al. (2001) used two-part implants in dogs, induced peri-implantitis and re-
placed the contaminated portion of the implant with a pristine part. Their study reported a
complete re-osseointegration and suggested that decontamination of the titanium surface is
of decisive importance for re-osseointegration. However, to date, human and animal studies
have failed to identify one chemotherapeutic agent as the gold standard for implant surface
decontamination (Claffey et al. 2008). Thus, the aim of this review was to search the litera-
ture for evidence regarding the most effective chemotherapeutic agent for the decontamina-
tion of infected titanium surfaces.

To re-establish titanium surface biocompatibility, it is imperative to remove the bacte-
rial deposits (Kozlovsky et al. 2006). Some treatments may achieve this goal but simultane-
ously render the titanium surface non-biocompatible. Conventional techniques used to clean
natural tooth surfaces usually cause irreversible and detrimental changes to the implant
(Burchard et al. 1991), thus compromising the biocompatibility (Schwarz et al. 2005). One
advantage of the chemical approach is that the titanium surface is not instrumented and
therefore runs only a minimal risk of damage (Strooker et al. 1998). Hydroxyapatite-coated
titanium surfaces treated with citric acid showed a greater number of attached fibroblasts
than sterile and untreated controls (Wittrig et al. 1992; Zablotsky et al. 1992b). CHX has also
been shown to promote gingival fibroblast attachment equivalent to that observed with
saline treatment (Burchard et al. 1991). Nevertheless, studies have shown that titanium sur-
faces may still suffer reduced biocompatibility after various chemical treatments. Zablotsky
et al. (1992b) and Wittrig et al. (1992) found that CHX, hydrogen peroxide and stannous fluo-
ride treatments resulted in significantly less fibroblast coverage of hydroxyapatite titanium
surfaces compared with sterile and untreated controls, respectively.

In the present review, only four eligible papers were identified. In vivo studies failed to
fulfill the eligibility criteria because the biofilm formation on these titanium surfaces could
not be standardized. Moreover, under such conditions, it is difficult to formulate a control
treatment or untreated controls. The evaluation parameters used in these types of studies
tend to be stated in terms of clinical outcomes such as the resolution of inflammation, prob-
ing depth, clinical attachment gain, radiographic data (such as bone fill) and histological parameters (such as re-osseointegration). To date, no in vivo studies have demonstrated a way to assess titanium surface decontamination in a “controlled” fashion.

To find evidence of the effectiveness of chemical treatments in decontaminating titanium surfaces, in vitro studies were reviewed as “a proof of principle”. In vitro studies provide the first measurable evidence that an investigational product might work in humans. Furthermore, in vitro tests allow for the inclusion of controls in the study without the addition of any moral or ethical concerns (Ulrey et al. 2005). Only when a specific treatment is solidly proven to be superior in vitro should in vivo studies, preferably randomized clinical trials, be initiated. The studies that were eligible for the present review did not go beyond the in vitro design, and all of them were considered to have a high potential level of bias.

Negative controls, or blanks, are substances such as sterile, deionized water, saline or other media that are expected to cause little or no change in the test system. All manipulations specified in the protocol (including removal of the tested solutions) should also be conducted using the negative control (Ulrey et al. 2005). The use of negative controls provides valuable information that is highly useful in interpreting the results obtained in in vivo and in vitro studies (Ulrey et al. 2005). Zablotsky et al. (1992a) and Dennison et al. (1994) evaluated both an untreated control and a control treatment against the various interventions. Whereas some interventions were significantly better than the untreated control, no intervention was better than the control treatment. Mouhyi et al. (2000) and Chin et al. (2007) only compared their treatments with an untreated control.

The most frequently used chemotherapeutic agents in the four studies included in this review were CHX and citric acid. The 0.12% CHX did not achieve a significant reduction of LPS on contaminated titanium surfaces as compared with untreated controls (Zablotsky et al. 1992a). Dennison et al. (1994) found that 0.12% CHX treatment removed 94.6% of the LPS from machined, contaminated implant surfaces, but less LPS (37.1%) from plasma-sprayed, contaminated implant surfaces. The effect of CHX was not significantly different from the water control treatment. Finally, Chin et al. (2007) found that 0.2% CHX was effective in killing multispecies biofilms and resulted in 79.5% fewer viable microorganisms compared with the untreated controls. On the other hand, CHX was only modestly effective in removing the biofilm.

Animal studies (Wetzel et al. 1999; Schou et al. 2003; You et al. 2007) have investigated the effects of a titanium surface treatment with CHX and saline. Low levels of re-osseointegration were achieved for non-machined implant surfaces (Claffey et al. 2008). These studies
did not assess decontamination of the implant surfaces, but the effect of CHX on clinical outcomes appears to be questionable. CHX has also been used for the treatment of peri-implant mucositis. A single professional irrigation of the sulci (Schenk et al. 1997; Porras et al. 2002) was not beneficial, but a self-administrated irrigation achieved significantly greater clinical improvement than rinsing (Felo et al. 1997).

In the study reported by Mouhyi et al. (2000), citric acid resulted in a cleaner titanium surface as observed by SEM than that associated with the untreated control. Citric acid was effective in the removal of LPS from titanium surfaces when compared with untreated controls, but it was not significantly more effective than saline or water (Zablotsky et al. 1992a; Dennison et al. 1994). The effectiveness of citric acid in LPS removal has been shown to be significantly greater on machined surfaces (90%) than on plasma-sprayed surfaces (34.4%) (Dennison et al. 1994). Zablotsky et al. (1992a) and Dennison et al. (1994) reported similar results. Citric acid showed no statistically significant differences in effectiveness as compared with water or saline. A possible explanation for this result is the small sample sizes used in both studies (three surfaces per treatment), which could be responsible for the lack of power and thus the lack of significant results.

An in vivo study in monkeys used citric acid as the chemotherapeutic agent for the treatment of TPS surface implants in combination with autogenous bone grafts and e-PTFE membranes (Schou et al. 2003). In that study, almost total bone fill was observed in all groups, and bone-to-implant contact ranged from 39% to 46%. Citric acid treatment did not differ significantly from CHX in that in vivo study. Khoury & Buchmann (2001) combined citric acid with CHX, H2O2 and saline to decontaminate implant surfaces before the placement of bone grafts and membranes. Neither of these studies was controlled, and decontamination was not assessed. Finally, Kolonidis et al. (2003) and Alhag et al. (2008) placed smooth and minimally rough (0.76 mm, on average) implants in dogs. They allowed some threads to protrude in the oral cavity to permit plaque accumulation and the development of peri-implant disease. The contaminated parts of each implant were treated using three different techniques: (1) swabbing with citric acid for 30 s, (2) cleansing with a toothbrush and saline for 1 min and (3) swabbing with 10% hydrogen peroxide for 1 min. Next, the treated implants and one pristine implant (control) were installed to the full implant length on the contralateral sides of the mandibles. The amount of osseointegration did not vary significantly, either between the different treatment modalities or in comparison with the new, sterile implant. These studies demonstrated that the method of decontamination used for the titanium surface might not
be a determining factor if the recipient site is healthy. Nevertheless, the implants used had a smooth or a minimally rough surface that facilitated the decontamination process (Dennis-son et al. 1994). Furthermore, in clinical reality, peri-implant tissues are likely to be inflamed, which can impair healing.

H$_2$O$_2$ has been used in clinical protocols for the treatment of infected implant surfaces (Mombelli & Lang 1998). In vitro studies of H$_2$O$_2$ decontamination have revealed conflicting results. Zablotsky et al. (1992a) showed that 3% H$_2$O$_2$ removes significantly more LPS from titanium surfaces when compared with untreated controls. In contrast, Mouhyi et al. (2000) found that 3% H$_2$O$_2$ had no obvious cleaning effect on contaminated titanium surfaces. In a clinical trial, Leonhardt et al. (2003) used H$_2$O$_2$ in combination with antibiotics and access surgery and observed healing in 58% of the implants.

Zablotsky et al. (1992a) and Dennison et al. (1994) utilized bacterial LPS to contaminate titanium surfaces. The rationale behind this choice was twofold. First, although the binding of endotoxin to the root surface appears to be weak (Nakib et al. 1982), Nelson et al. (1997) observed that LPS had a high affinity for titanium biomaterials. Further, endotoxin is a characteristic component of the cell wall of gram-negative bacteria and it plays a significant role in the binding process of these bacteria and in initiation of the host response. LPS from oral bacteria has a marked effect on most types of cells found in the periodontal tissues, including macrophages, lymphocytes, fibroblasts and osteoblasts (Wilson 1995). Bacterial endotoxin has been shown to inhibit fibroblastic growth and attachment to root surfaces (Layman & Diedrich 1987). Zablotsky et al. (1992a) showed that the removal of LPS from hydroxyapatite-coated titanium surface promoted more effective human gingival fibroblast growth and attachment compared with the untreated control. Whether this effect also occurs on uncoated titanium surfaces remains unknown. The results reported by Nouneh et al. (2001) indicated that the presence of LPS did not significantly alter osteoblast attachment to titanium or titanium alloy surfaces, irrespective of whether the exposure occurred before or after cellular adherence. The biological and clinical significance of removing bacterial components like LPS require further validation. In addition, the use of LPS removal as an outcome variable might not adequately represent the overall ability of the tested chemotherapeutic agents to remove the biofilm and vice versa. In our opinion, it is more clinically relevant to grow biofilms on titanium surfaces to test various chemical treatments. Furthermore, this approach can provide information regarding both the killing and removal abilities of these agents. The only study to investigate the killing capacities of antimicrobials was that reported by Chin et al.
(2007). The greatest shortcoming of that study was the use of machined titanium surfaces. Machined titanium surfaces are mostly limited to the neck of the implants, but peri-implant disease often involves exposure of the rough titanium surface to the oral environment.

Quantifiable results are fundamental for effective comparisons of study outcomes (Field et al. 2010) and therefore they reflect the quality of the study. Mouhyi et al. (2000) used titanium surfaces that were contaminated biologically by placing the discs in dentures in the mouths of volunteers. Further, they used SEM to evaluate the cleaning effect of the different chemicals. This method is clearly not quantitative and thus does not allow us to draw any definitive conclusions.

The real incidence of peri-implantitis is probably underestimated (Esposito et al. 2007). The high number of dental implants placed and their longer follow-up periods will inevitably lead to more cases of diagnosed peri-implantitis. Thus, the need for efficient treatment and further maintenance of successfully treated implants will increase in the near future. The interventions tested in the various studies presented herein are mostly empirical, and the study outcomes are inconsistent and unpredictable. This finding does not mean that all current treatments are ineffective (Esposito et al. 2008), but there is still no consensus among clinicians regarding the best treatment. In our opinion, a systematic approach to the treatment of contaminated implant surfaces should be initiated. The available treatment modalities should be categorized and evaluated separately in a controlled manner. Reviewing the literature for this type of studies on chemical decontamination of titanium surfaces was rather disappointing. Considering the number of studies that have been published on the technical aspects and aesthetic outcomes of implant surgery, it is striking that so little controlled research has been undertaken to determine how the titanium implants should be maintained in order to reduce the chances of biological complications (perimucositis and peri-implantitis) and further how to treat the titanium surfaces in the event of such complications. Additional work in this area of research is imperative. Finally, the greatest challenge will be to determine the treatment protocol that best balances decontamination (Persson et al. 2001) and re-establishment of the biocompatibility of the titanium surface with the stimulation and promotion of healing in peri-implant tissues (Kolonidis et al. 2003; Alhag et al. 2008).
Conclusion

The data reported on the efficacy of chemotherapeutic agents for the treatment of contaminated titanium surfaces are scarce, which precludes the generation of any firm conclusions. Based on the limited available evidence, we cautiously conclude that citric acid seems to be the chemotherapeutic agent with the highest potential for the removal of biofilms from contaminated titanium surfaces \textit{in vitro}, although complete removal was not achieved. To date, the killing effect of citric acid has not been investigated on titanium surfaces.

\textit{Implications for future research}

Owing to the limited and weak evidence that is available, further research is required. Future studies should include an appropriate negative control, and titanium surfaces should be preferably contaminated with bacterial biofilms rather than bacterial components such as LPS. Additionally, the assessment of surface decontamination should involve quantification of the residual biofilm. The results obtained using rough titanium surfaces are more clinically relevant and increase the applicability of the findings. Finally, \textit{in vivo} studies should be performed to test the \textit{in vitro} findings and to establish an evidence-based protocol for the treatment of peri-implant diseases.

Acknowledgements

This paper was prepared as a part of the obligation of the first author to fulfill the requirements of the ACTA master’s program in Periodontology and Implantology. \textit{Conflict of interest and source of funding statement}: The authors declare that they have no conflict of interest. This study was self-funded by the authors and their institutions.

\textit{Authors’ contributions:}

\textit{V.I. Ntouka} contributed to the conception, design, acquisition, analysis, interpretation of data, drafted the manuscript.

\textit{D.E. Slot} contributed to the design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.

\textit{A. Louropoulou} contributed to the conception, design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.
G.A. van der Weijden contributed to the conception, design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.

All authors gave final approval and agree to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
References


Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds


Ulrey A, Curren R, Raabe H. (2005) Applying Good Laboratory Practices (GLPs) to in vitro studies, one laboratory’s perspective. Presented at the 6th World Congress on Alternatives and Animal Use in the Life Sciences, August 21–26, Berlin, Germany.


The effect of chemotherapeutic agents on...


* Studies included in the review

Table 1. Overview of the studies that were excluded after complete reading and the reason for exclusion

<table>
<thead>
<tr>
<th>Reason for rejection</th>
<th>Author(s) (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination of mechanical and chemical treatment</td>
<td>Schwarz et al. (2005)</td>
</tr>
<tr>
<td>Surface preparation, not chemical treatment</td>
<td>Kilpadi et al. (2000)</td>
</tr>
<tr>
<td>Hydroxyapatite-coated titanium strips (not a titanium surface)</td>
<td>Wittrig et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Zablotsky et al. (1992b)</td>
</tr>
<tr>
<td></td>
<td>Zablotsky et al. (1992c)</td>
</tr>
<tr>
<td>Non-contaminated titanium surfaces</td>
<td>Burchard et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>Kozlovsky et al. (2006)</td>
</tr>
<tr>
<td>Not controlled and non-standardized biofilm growth (failed implants)</td>
<td>Mouhyi et al. (1998)</td>
</tr>
</tbody>
</table>

Figure 1. Database search and literature selection.
**Table 2. Details of the selected studies**

<table>
<thead>
<tr>
<th>Author (year) Title</th>
<th>Surface</th>
<th># of surfaces treated</th>
<th>Treatment</th>
<th>Outcome measure</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zablotsky et al. (1992a) Detoxification of endotoxin-contaminated titanium and hydroxyapatite-coated surfaces utilizing various chemotherapeutic and mechanical modalities</td>
<td>Contaminated with <em>Eschericia coli</em> LPS Grit-blasted titanium alloy strips</td>
<td>3</td>
<td>Chlorhexidine gluconate (0.12%) Stannous fluoride (1.64%) Tetracycline HCl (50 mg/ml) Chloramine T (1%) Saline (3%) Hydrogen peroxide (3%) Citric acid (pH 1)</td>
<td>Residual lipopolysaccharide levels measured by liquid scintillation spectrometry</td>
<td>Citric acid was significantly superior for the removal of LPS from grit-blasted titanium alloy when compared with the untreated control. Citric acid treatment resulted in the lowest amount of residual LPS, but when compared with saline, it failed to reach statistical significance</td>
</tr>
<tr>
<td>Dennison et al. (1994) Contaminated implant surfaces: an <em>in vitro</em> comparison of implant surface coating and treatment modalities for decontamination</td>
<td>Contaminated with <em>Porphyromonas gingivalis</em> LPS Machined and plasma-sprayed titanium implants</td>
<td>6</td>
<td>d-H₂O Saturated citric acid Chlorhexidine gluconate (0.12%)</td>
<td>Radioactive endotoxin (radioimmunooassay)</td>
<td>Citric acid and CHX were superior to an untreated control and equally effective as water for machined and plasma-sprayed titanium surfaces</td>
</tr>
<tr>
<td>Author (year)</td>
<td>Title</td>
<td>Surface</td>
<td># of surfaces treated</td>
<td>Treatment</td>
<td>Outcome measure</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-----------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Mouhyi et al. (2000)</td>
<td>Re-establishment of the atomic composition and the oxide structure of contaminated titanium surfaces by means of carbon dioxide laser and hydrogen peroxide: An in vitro study</td>
<td>Contaminated by placement on dentures in volunteer patients commercially available pure titanium foils textured surface</td>
<td>2</td>
<td>Untreated control</td>
<td>Observations using a Scanning electron microscope</td>
</tr>
<tr>
<td>Chin et al. (2007)</td>
<td>Biofilm formation on surface characterized micro-implants for skeletal anchorage in orthodontics.</td>
<td>Contaminated with saliva biofilm Four commercially available, self-tapping micro-implants (Two pure titanium, two titanium alloy) machined surface</td>
<td>12</td>
<td>Untreated control</td>
<td>CLSM analysis of dispersed biofilms</td>
</tr>
</tbody>
</table>

LPS, lipopolysaccharide; CSLM, confocal laser scanning microscopy.
Table 3. Methodological quality scores of the selected studies

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>External validity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Representative surface material*</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Validation of the model</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Validation of the evaluation method</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Reproducibility data provided*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Internal validity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random treatment allocation*</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Blinded to examiner</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Blinding during statistical analysis</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Preparation, manipulation and treatment of the surface identical, except for the intervention*</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Statistical validity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size and power calculation</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Point estimates presented for primary outcome measurements</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Measures of variability presented for the primary outcome</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Statistical analysis*</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Author’s estimated risk of bias</strong></td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

*Items used to estimate potential risk of bias.

?, not specified/unclear; +, yes; –, no.
Table 4. Mean residual LPS counts on grit-blasted titanium alloy strips, and levels of significance for the treatments compared with the untreated control (adapted from Zablotsky et al. 1992a)

<table>
<thead>
<tr>
<th>Treatment</th>
<th># titanium strips</th>
<th>LPS counts/min/mm²</th>
<th>% Removal relative to untreated control</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnF₂ (1.64%)</td>
<td>3</td>
<td>302*</td>
<td>NA</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Untreated control</td>
<td>3</td>
<td>197</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CHX (0.12%)</td>
<td>3</td>
<td>170</td>
<td>13.7%</td>
<td>NS</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3</td>
<td>141</td>
<td>28.4%</td>
<td>NS</td>
</tr>
<tr>
<td>H₂O₂ (3%)</td>
<td>3</td>
<td>108</td>
<td>45.2%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Saline</td>
<td>3</td>
<td>98</td>
<td>50.2%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Chloramine T</td>
<td>3</td>
<td>86</td>
<td>56.3%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3</td>
<td>68</td>
<td>65.5%</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Significantly greater amounts of LPS than in the untreated control (p<0.05).
◇, calculation by the authors of this review; NA, not applicable; NS, not significant;
■, untreated and saline-treated controls; LPS, lipopolysaccharide.
Table 5. Reduction of endotoxin level relative to baseline values on machined and plasma-sprayed titanium surfaces, and level of significance for the treatments compared with water (adapted from Dennison et al. 1994)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Machined</th>
<th>Plasma sprayed</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
</tr>
<tr>
<td>d-H₂O</td>
<td>3</td>
<td>92.4*</td>
<td>3</td>
</tr>
<tr>
<td>CHX</td>
<td>3</td>
<td>94.6*</td>
<td>3</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3</td>
<td>90*</td>
<td>3</td>
</tr>
</tbody>
</table>

*Significant compared with baseline.
■, water-treated control; NA, not applicable; NS, not significant.

Table 6. Mean percentage of viable organisms remaining on machined titanium surfaces after treatment, and level of significance compared with untreated controls (adapted from Chin et al. 2007)

<table>
<thead>
<tr>
<th>Treatment</th>
<th># Titanium surfaces</th>
<th>% Mean (SD)</th>
<th>Significance levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control ■</td>
<td>12</td>
<td>57 (4.5)</td>
<td>NA</td>
</tr>
<tr>
<td>CHX (0.2%)</td>
<td>12</td>
<td>11.7 (4.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NaF (0.05%)</td>
<td>12</td>
<td>10.5 (5.3)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

NA, not applicable.
■, untreated control.
## Table 7. Summary of the outcomes of the included papers, treatments and comparisons

<table>
<thead>
<tr>
<th>Author</th>
<th>Outcome parameter</th>
<th>Comparison</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zablotsky et al. (1992a)</td>
<td>Removal of LPS</td>
<td>Untreated</td>
<td>CHX (0.12%)</td>
</tr>
<tr>
<td>Dennison et al. (1994)</td>
<td>Removal of LPS</td>
<td>Saline</td>
<td>CHX (0.2%)</td>
</tr>
<tr>
<td>Mouhyi et al. (2000)</td>
<td>Clean surface at SEM</td>
<td>Untreated</td>
<td>SnF₂ (1.64%)</td>
</tr>
<tr>
<td>Chin et al. (2007)</td>
<td>Removal</td>
<td>d-H₂O</td>
<td>Tetracycline</td>
</tr>
<tr>
<td></td>
<td>Killing</td>
<td>Untreated</td>
<td>Chloramine (1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saline (3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H₂O₂ (3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Citric acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NaF (0.055%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Citric acid and H₂O₂</td>
</tr>
</tbody>
</table>

+, statistically significant difference; −, no significant difference; ■, not applicable; ○, no statistical analysis was performed; LPS, lipopolysaccharide; SEM, scanning electron microscopy. This reflects a descriptive summary.